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Development and optimization of *Curcuma longa* Linn. oleoresin non-aqueous gel for transdermal delivery

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ABSTRACT

A long-term oral administration of NSAID and DMARD on rheumatoid arthritis (RA) treatment may cause gastritis, kidney, and cardiovascular disorder. One of the alternative therapies that have been investigated is by using herbal medicine such as *Curcuma longa* Linn. which contains curcumin and essential oils. Even though both compounds are quite effective in treating RA, poor aqueous solubility and low intestinal absorption limit their oral bioavailability. To overcome these drawbacks, transdermal delivery was chosen as an alternative route of administration. This study was aimed to formulate the *Curcuma longa* Linn. oleoresin into a transdermal non-aqueous gel system using Carbopol 934 and low substituted hydroxypropyl cellulose (4.25:0.75 %) as the gelling agent. In this study, multiple solvents (PEG 400, PG, glycerin, ethanol, and tween 20) were used in the system. The solvents were chosen based on their ability to dissolve the gelling agents. Optimization was done using a simplex lattice design based on the physical characteristics (viscosity, pH, spreadability, and adhesivity) of the prepared gel. The system with the optimum concentration of PEG 400 and PG was then observed for its stability and *in vitro* transport through snakeskin membrane using Franz diffusion cell with PBS pH 7.4 as acceptor medium. The optimal formula was comprised of 75% PEG and 25% PG which has a viscosity of 6.34±0.19 dPa.s, adhesivity of 6.05±0.11 seconds, pH of 5.16±0.09, spreadability of 6.94±0.06 cm, and quite stable after freeze-thaw cycling test, whilst around 26.85% curcumin was diffused through the membrane (flux = 0.084 $mg.cm^{-2}$) after 2 hours. It can be concluded that the *Curcuma longa* Linn. oleoresin can be formulated into a non-aqueous gel system, which showed a fair gel physical characteristic with good stability and ability to permeate across the skin membrane, and is promising to be further developed as an alternative for RA treatment.

Keywords: *Curcuma longa* Linn., Non-aqueous gel, Transdermal, Rheumatoid arthritis

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INTRODUCTION

Rheumatoid Arthritis (RA) is a chronic, progressive inflammatory autoimmune disease that impacts the musculoskeletal system and is commonly characterized by aggressive and symmetric polyarthritis (Chabib et al., 2018; Dai et al., 2018; Pourhabibi-Zarandi et al., 2021). Even RA has an extensive history, its etiology is still unknown. Many researchers believe that RA is resulted from genetics, immune system, and environmental interaction (Chabib et al., 2018). Many environmental factors such as hormones, diet, infections, and tobacco exposure can increase the risk. About 0.8% of the global population is suffering from RA, whilst its prevalence in Indonesia is around 11.9% (Olwin, 2009; Rudan et al., 2015). Though the prevalence is low, RA can seriously cause systemic complications and affect the patient's quality of life which leads to economic burden (Chabib et al., 2018). Therefore, proper therapy is required in treating RA to avoid an undesirable outcome.

RA therapy is focusing on controlling the inflammatory disease, which could alleviate pain by using non-steroidal anti-inflammatory drugs (NSAID) such as piroxicam, ibuprofen, or diclofenac sodium and disease-modifying anti-rheumatic drugs (DMARD) like hydroxychloroquine, methotrexate, or sulfasalazine (Chabib et al., 2018; Dai et al., 2018; Pourhabibi-Zarandi et al., 2021). Those drugs may provide symptomatic relief and slow the disease progression. However, a long-term oral administration of NSAID and DMARD (1-2 months) may cause gastritis, stomach ulcer, kidney and cardiovascular disorder (Dudics et al., 2018; Nagai et al., 2015). One of the alternative therapies that have been investigated by many researchers on treating RA is using herbal medicine which is known to possess an anti-inflammatory activity such as *Curcuma longa* Linn. or turmeric (Dai et al., 2018; Pourhabibi-Zarandi et al., 2021).

The extraction of *Curcuma longa* Linn. using organic solvent could yield various extracts such as a dry extract, oil, and oleoresin of *Curcuma longa* Linn. The oleoresin itself is commonly produced by extracting the turmeric dry powder using ethyl acetate, acetone, or 95% ethanol. The solvent is then evaporated by distillation until a brown viscous liquid with a distinctive turmeric odor is obtained (Haldar et al., 2015). *Curcuma longa* Linn. consists of curcuminoids and essential oils such as α-turmerone (42.6%), β-turmerone (16%), and ar-turmerone (12.9%) (Avanço et al., 2017). However, the oleoresin product consists of the highest amount of both curcuminoids (20-30%) and essential oils (30-33%) compare to the other *Curcuma Longa* Linn products (Bampidis et al., 2020). Intraperitoneal administration of essential oil-depleted turmeric dry extract which contains 41% curcuminoids with no detection of essential oil was only able to decrease the joint inflammation by 72%, compared to crude or refined turmeric essential oil product which show drastically decreased in joint swelling (90-100%)(Funk et al., 2006, 2010). However, when administered orally, the anti-arthritic efficacy of both products reduced to 20% (Funk et al., 2010). Both curcumin and turmeric essential oils can inhibit the activation of the NF-κB transcription factor. NF-κB activation can increase the expression of cytokine genes (TNF-R, IL-1), chemokines (MCP-1), and COX-2 which are the inflammatory mediator on RA (Dai et al., 2018; Dudics et al., 2018). Therefore, oleoresin which contains both curcuminoids and essential oils of turmeric could be potential in reducing joint inflammation.

Unfortunately, even though curcumin and turmeric essential oils are quite effective in treating RA, this compound has a limitation when used orally. Curcumin is known to have low oral bioavailability due to its low solubility, rapid systemic clearance, inadequate tissue absorption, and it degrades at alkaline pH values (Paolino et al., 2016). Whilst turmeric essential oil was comprised of various volatile terpenoids that have hydrophobic nature which limits its oral absorption (Funk et al., 2010). Therefore, in this study, the *Curcuma Longa* Linn. oleoresin was administered by the transdermal route to overcome those drawbacks. With high lipophilicity (log P = 3.6) and low molecular weight (380 Da) made curcumin suitable to be delivered via transdermal route, while essential oils are known to be used as a skin penetration enhancer and promote dermal absorption via increasing drug partition through skin layer or disrupting the skin morphology (Herman &

Herman, 2015; Songkro et al., 2008; Yousef et al., 2019). To be effective, the drug delivered via the transdermal layer has to be applied on the skin and pass the skin layer before entering the systemic circulation. Human skin consists of 3 layers with different structures and characteristics. The stratum corneum, the outermost of the skin layer, is known to be the biggest obstacle in the transdermal delivery system. The stratum corneum consists of 5-7 stacks of skin cells (corneocytes), making it hard to be penetrated by drugs (Aggarwal et al., 2014; Cevc & Vierl, 2010; Gannu et al., 2007; Kamel, 2016). Using a suitable transdermal formulation is one way to widen the pathway across the stratum corneum.

This study was aimed to formulate the *Curcuma longa* Linn. oleoresin into a transdermal non-aqueous gel (NAG) system using Carbopol 934 and Low-substituted Hydroxy Propyl Cellulose (LHPC) as the gelling agent. The use of non-aqueous gel to deliver *Curcuma longa* Linn. oleoresin has never been done before. The non-aqueous gel was chosen as the carrier since it contains a lot of organic solvents (such as ethanol and surfactants). The organic solvents were used to dissolve the gelling agent in this system without involving water in the gelation process (Chow et al., 2008; Lenhart et al., 2007). Besides working as a solvent, ethanol and surfactants can also work as penetration enhancers on the transdermal formulation. Those ingredients are known to have the ability to modify the stratum corneum structure (Cevc & Vierl, 2010). LHPC is a polymer that is commonly used in non-aqueous gel preparation since it can easily dissolve in organic solvents, whilst Carbopol is a crosslinked polyacrylic acid polymer with a high molecular weight and hydrophilic characteristic. Though this polymer is well known to form gel structure in water with controlled pH and temperature, Carbopol can also form gel while using ethanol, glycerol, propylene glycol (PG), and polyethylene glycol (PEG) 400 as the solvents without neutralization, so it can be used in non-aqueous gel formulation (Varges et al., 2019).

In this study, multiple solvents (PEG 400, PG, glycerin, ethanol, and tween 20) were used in the system. The solvents were chosen based on their ability to dissolve the gelling agents (preliminary study data not shown). The previous study showed that the choice and amount of the solvents used in the formulation could affect the gel consistency (Slater, 1990; Varges et al., 2019), therefore it became the main interest in this research. Optimization was done using a simplex lattice design based on the physical characteristics of the prepared gel. A system with the optimum concentration of PEG 400 and PG was then observed for its *in vitro* transport through snakeskin membrane as a first step to prove that it can be transported via the transdermal route.

MATERIALS AND METHODS

Materials

The *Curcuma longa* L. oleoresins used for this study was purchased from Javaplant, Indonesia (curcuminoids content is 19.1%). Other materials used in this research were curcumin standard (Merck), PBS buffer pH 7.4 (Biogear), propylene glycol, PEG 400, tween 20, glycerin, and carbopol 934 were obtained from Bratachem, ethanol pro analysis (Merk), low substituted hydroxypropyl cellulose (LHPC) was purchased from Shandong Head Co.ltd. Shed snakeskin (Phyton Regius 2 mm in thickness), Spectrophotometer UV-Vis Genesys 10S (Thermo Fisher Scientific), Franz diffusion cell vertical type were used in diffusion study, whilst viscometer Rion Vt-6 is used to obtain the non-aqueous gel (NAG) viscosity.

Methods

Optimization of PEG 400 and Propylene glycol using Simplex Lattice Design

The composition of PEG 400 and PG as solvent and penetration enhancer in a non-aqueous gel formulation was optimized using a simplex lattice design mixture, conducted with Design expert version 10.0.3 software. Simplex lattice design was used for screening the effect of formulation variables on gel physical properties such as pH, viscosity, adhesivity, and spreadability. The layout of the simplex lattice design is depicted in Table 1.

Table 1. The layout of simplex lattice design in nonaqueous gel formulation

Ingredients		Run							
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Variables (%)	PEG 400(A)	0	100	100	25	50	50	75	0
	PG (B)	100	0	0	75	50	50	25	100
Variables (gram)	PEG 400 (A)	0	63	63	15.75	31.5	31.5	47.25	0
	PG (B)	63	0	0	47.25	31.5	31.5	15.75	63
Solvents (gram)	Ethanol	10							
	Glycerin	10							
	Tween 20	10							
Gelling agent (gram)	L-HPC	4.25							
	Carbopol	0.75							
Active ingredients (gram)	*Curcuma Longa* Linn. Oleoresin	2							
Total (gram)		100							

Preparation of *Curcuma Longa* Linn. oleoresins nonaqueous gel (NAG)

All of the ingredients provided in Table 1 were weighed accurately. Firstly, L-HPC was slowly dispersed into a vehicle mixture consisting of tween 20, glycerin, PEG 400, and PG (mixture A), and this mixture was allowed to thicken at room temperature using an overhead stirrer (IKA RW 20, USA) at 900 rpm for 2 h. Carbopol 934 was slowly added in small aliquots into the ethanol in a separate beaker with stirring using a magnetic stirrer (300 rpm) to aid gelation until a clear gel was formed (mixture B). Mixture A was then slowly added into mixture B and to this mixture, *Curcuma Longa* Linn. oleoresin (2g) was added until yellow clear gel was formed. The agitation was continued for another hour to ensure the complete swelling of gel before any testing.

Evaluation of NAG preparation

Measurement of pH

One gram of each formulation was diluted using 10 mL of distilled water, then the pH of the formulation was evaluated using calibrated pH meter (Hanna, HI 2210). This test was done in triplicates.

Measurement of viscosity

The viscosity of all formulations was determined using Viscotester Rion VT-6. Each formulation was weighed accurately for 150 g and was added to the cup. The measurement was done using spindle no 3 at room temperature. The reading on the display was then written as the viscosity of the sample. This test was done in triplicates.

Spreadability study

The spreadability of all formulations was determined by a modified apparatus. A total amount of 0.5 g of gel was placed in between two glasses for one minute, then the geometric diameters (vertical, horizontal, and diagonal) were measured. The test was then followed by the addition of 150 grams of weight on the top of the glass and was left for one minute. The weight was then removed and the spreading diameters of gel were observed. This test was done in triplicates.

Adhesion test

The adhesive properties of all formulations were carried out by a modified apparatus. A total amount of 0.5 g of gel was placed in between two glass slides and then 500 g of weight was placed on the top of slides for 5 minutes to compress the sample to a uniform thickness. One end of the

slide was attached to the clip which was tied into 80 g of weight. The 500 g weight was then removed and the time (seconds) required to separate the two slides, was taken as a measure of adhesivity. This test was done in triplicates.

Accelerated stability study

The stability of the optimized formulation was determined using the freeze-thaw cycling method. The formulation was subjected to freezing temperature (approximately -15^{o}C) for 24 hours and then store at room temperature for the thawing process for another 24 hours (1 cycle). The formulation is then analyzed for significant changes in physical properties (pH, viscosity, spreadability, and adhesivity) after 6 cycles.

In-vitro diffusion study

In-vitro diffusion study of optimized formulation was conducted using vertical type Franz diffusion cell. The receptor chamber (volume of 23 mL) was filled with phosphate buffer saline (pH of 7.4) and stirred with the help magnetic stirrer. The temperature of the cell was maintained at 37^{o}C using a circulating water bath (Memmert, Germany). Shed snakeskin (2 mm in thickness) was used as a diffusion membrane, which was placed between the donor and receptor chamber. The diameter of the diffusional area was 3.49 cm (total area of diffusion was 9.61 cm^2). Optimized NAG formulations (2 g) were placed on the donor chamber and 1 g of oleoresins was used as a comparison to compare the effectiveness of the formulation. At pre-determined time intervals (15, 30, 45, 60, 90, and 120 minutes), two (2) mL of sample was withdrawn from the receptor chamber and replaced with the same volume of the medium. All samples were analyzed for curcumin content spectrophotometrically at 430 nm using curcumin standard curve with PBS pH 7.4 as solvent. The initial amount of curcumin from both oleoresin and optimized NAG were also determined spectrophotometrically at 425 nm using a curcumin standard curve with ethanol as solvent.

Data Analysis

Simplex lattice mixture design was used to determine the optimum concentration of PEG 400 (A) and propylene glycol (B) as independent variables in nonaqueous gel formulation toward the parameters which are viscosity (Y1), pH (Y2), adhesivity (Y3), and spreadability (Y4)**.** The best-fitted model selected from linear, quadratic, cubic, or quartic was used to explain the relation of each independent variable and parameters or response. This model was determined using statistical parameters such as p-value of ANOVA, lack of fit, or adequate precision. This analysis also results in an equation from the best-fitted model which is then used to explain the contribution of each variable toward the response. Also, a contour plot for each response was generated by Design Expert ver 10.0.3 for a better explanation. After model analysis, the optimization menu was used to select the best formula based on the desirability value of each solution recommended by the software. Statistical analysis was performed using paired t-test to test the difference between the means of each response before and after the stability test. Data were considered statistically different at a p-value <0.05.

RESULT AND DISCUSSION

Optimization of PEG 400 and Propylene glycol using Simplex Lattice Design

The selection of appropriate formulation components is an important factor in the preparation of stable and effective nonaqueous gel formulation. The gelation process of polymers occurred differently depending on the solvent type and loading, and thus will affect the release and penetration ability of active pharmaceutical ingredients from the formulation into the skin. PEG 400 and propylene glycol have been used in a range of topical and transdermal formulations, as a permeation enhancer, and their use as a solvent for aiding the dispersion of polymeric gelling agents such as hydroxypropyl cellulose and Carbopol has been studied before. Differences in physical properties between PEG 400 and propylene glycol such as viscosity resulted in the variability in

physical quality attributes of the formulation. Therefore, an optimization of PEG 400 and PG concentration is needed to further evaluate its effect on the physical properties of the result in the nonaqueous gel.

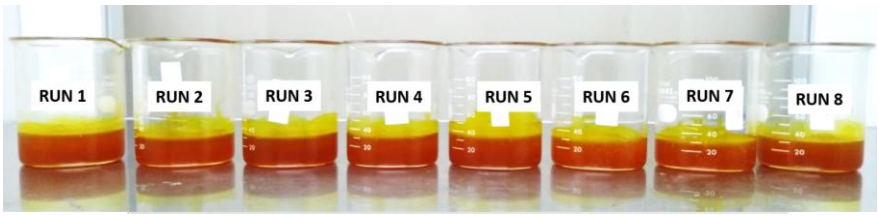

Figure 1. The visual appearance of *Curcuma Longa* Linn. Oleoresin non-aqueous gel

Table 2. Characteristics of all 8 runs of *Curcuma Longa* Linn. oleoresin Non-aqueous gels based on four parameters

RUN	Independent variable		Response variable (mean±SD (n=3))			
	PEG 400 (A) (%)	PG (B) (%)	Viscosity (Y1) d.pas	pH (Y2)	Adhesivity (Y3) (seconds)	Spreadability (Y4) (cm)
1	0	100	7.67±0.115	4.78±0.237	0.80±0.168	8.47±0.040
2	100	0	5.53±0.404	5.26±0.012	1.77±0.563	7.54±0.846
3	100	0	5.30±0.100	5.22±0.012	1.30±0.165	7.57±0.119
4	25	75	6.00±0.100	5.30±0.020	0.77±0.115	7.93±0.289
5	50	50	6.8±0.100	5.09±0.012	1.82±0.935	7.03±0.160
6	50	50	6.53±0.152	5.03±0.005	2.49±2.55	7.21±0.278
7	75	25	6.43±0.115	5.10±0.005	6.64±0.916	6.59±0.358
8	0	100	7.80±0.100	4.66±0.190	1.32±0.555	7.77±0.202

Optimization of two variables using simplex lattice design resulted in 8 formulations (runs) with different concentrations of PEG 400 and propylene glycol. The goal of the optimization experiments was to formulate nonaqueous gel with acceptable appearance, viscosity, pH, spreadability, and adhesivity. All the prepared formulations resulted in a clear, yellow, smooth, and homogeneous gel texture as depicted in Figure 1. Based on Table 3 the pH value of all the formulations ranged from 4.66 to 5.30, which are inside the range of the physiological pH of healthy skin (4.1-5.8) (Proksch, 2018). Nonaqueous gel prepared from a varied concentration of PEG 400 and propylene glycol has a viscosity ranging from 5.30 to 7.80 d.pas or 530 to 780 cps which is very thin for semisolid preparation which usually have a viscosity of around 2000 to 4000 cps (Nurman et al., 2019). Viscosity is an important factor for semisolid formulations as it may affect application on the skin (spreadability and adhesivity) and release of drug from the gel by influencing the diffusion rate. Easily spreadable and good adhesion to the skin to prevent loss of drug during application is an important characteristic of the transdermal formulation. Although all formulations have low viscosity, most formulations still have good adhesivity. Topical formulations were considered to have good spreadability if the diameter of the circle after spreading of the gel is in the range of 5 to 7 cm, and good adhesivity if the time required for separating two slides with formulation placed in between them is more than 1 second (Garg et al., 2002; Nurman et al., 2019; Yusuf et al., 2017). To evaluate the effect of variability of PEG 400 and propylene glycol on all parameters, data listed in Table 2 were subjected to analysis of the best-fitted model, polynomial equation, and contour plot using Design-Expert software version 10.0.3. It was observed that the best-fitted model was cubic and quartic and comparative p-value for ANOVA, R-

squared (R^2), and lack of fit along with the equation of actual generated for each response are given in Table 3, and contour plot of the model which depicts the effect of two variables on each measured response is shown in Figure 2.

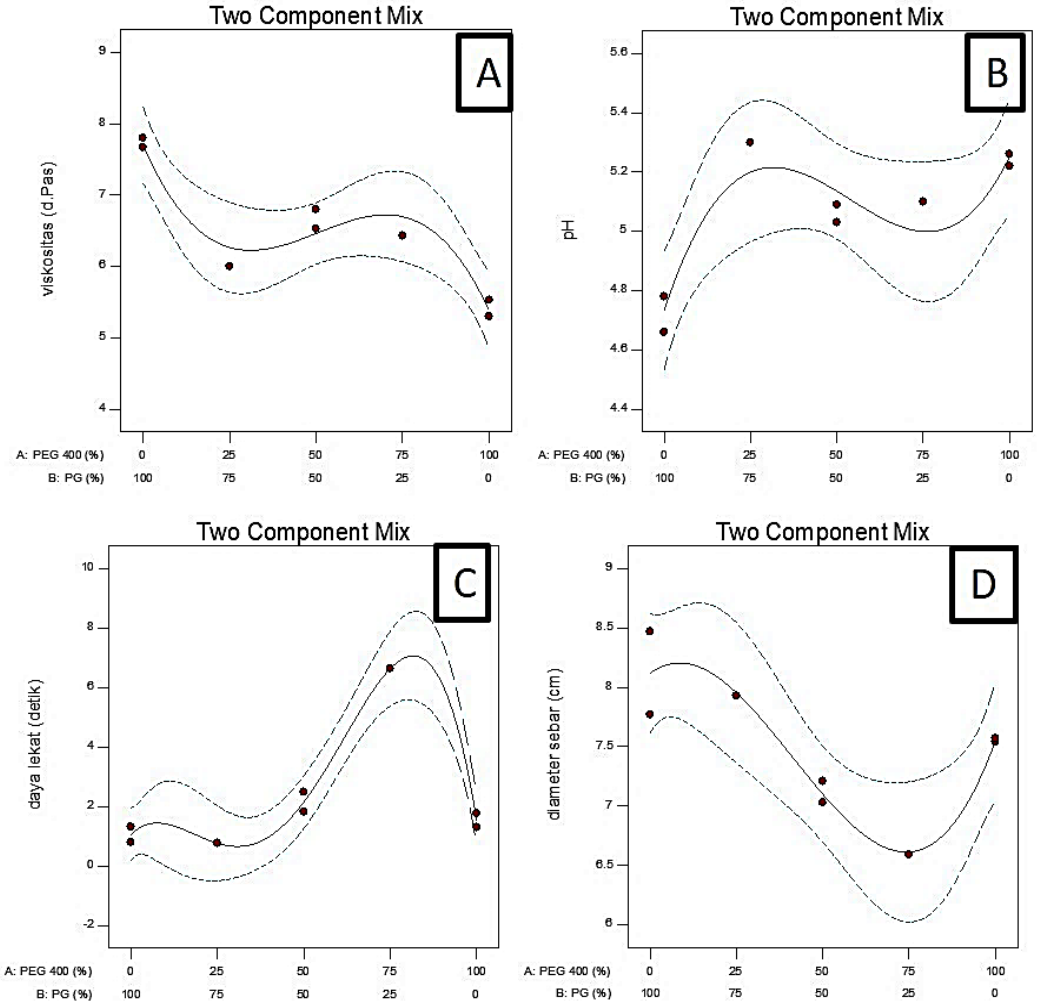

Figure 2. Contour plot of the model representing the effect of PEG 400 (A) and PG (B) on the measured response. A on viscosity (Y1), B on pH (Y2), C on adhesivity (Y3), D on spreadability (Y4)

As shown in Table 3 and Figure 2, the cubic model was a best-fitted model to explain the effect of both independent variables on viscosity, pH, and spreadability because the p-value of the model was less than 0.05, lack of fit analysis of the model was not significant ($p > 0.05$), and high value of R-squared (R^2) (Nikzade et al., 2012). Meanwhile, the effect of the independent variable on adhesivity was best explained with the quartic model. Although the model p-value for adhesivity was significant and the p-value of lack of fit cannot be calculated due to the limitation of data freedom of degree for a quartic model with 2 variables, the R-squared value of this model is very high (0.9817) which indicate a strong relationship between the model and the dependent variable. Another statistic parameter, which is adequate precision could also be used to indicate that the fitted model can be used to navigate the design space. To do so, the ratio of adequate precision must be greater than 4 (Diedericks & Jideani, 2015). As shown in Table 3, the quartic model of adhesivity has adequate precision of 18.757, therefore this parameter can be used for optimization. Equation of actual shown at Table 3 reflects the quantitative effect of independent variables and their

interaction on the response. The positive sign of coefficient signifies the synergistic effect while the negative sign signifies the opposing effect on the response. The larger coefficient value of the independent variable indicates their substantial effect on the response (Yadav et al., 2020). Based on the equation, both PEG 400 and PG have a positive sign of coefficient therefore an increase in their concentration will increase the value of each response. PEG 400 was the dominant factor in increasing the pH and adhesivity of nonaqueous gel, while PG was the dominant factor in affecting the spreadability and viscosity value. PEG 400 is a more viscous liquid compared to PG. PEG 400 has a viscosity of 114.5 mPa.s at 20ºC while PG has a viscosity of 60.61 mPa.s (Sagdeev et al., 2017; Sequeira et al., 2019). Solution of 10% PEG 400 in water has pH of 5, and the pH will increase proportionally with PEG 400 concentration, therefore an increase of PEG 400 concentration will result in a more basic gel and its thicker nature will increase the adhesivity of the gel (Guilminot et al., 2002).

Table 3. Summary of the fitted model and polynomial equation between independent variables and response

Respons (y)	**ANOVA (p-value)**	***Equation of actual***	***Lack of fit (p-value)***	**R^2**
Viscosity (Y1)	Cubic (p=0.0050)	$Y= 0.054A+0.077B-3.106 x10^{-5} AB+8.480 x10^{-6}AB(A-B)$	Not significant (p=0.0531)	0.9480
pH (Y2)	Cubic (p=0.0238)	$Y=0.053A+0.047B+5.71 x10^{-5}AB-2.45 x10^{-6}AB(A-B)$	Not significant (p=0.0518)	0.8851
Adhesivity (Y3)	Quartic (p=0.0061)	$Y=0.015A+0.011B+3.43x10^{-4} AB+3.004 x10^{-5}AB(A-B)+3.764AB(A-B)^2$	N/A* Adec precision (18.757)	0.9817
Spreadability (Y4)	Cubic (p=0.0229)	$Y=0.075A+0.081B-2.931 x10^{-4}AB-5.640 x10^{-6}AB(A-B)$	Not significant (p=0.8907)	0.8872

PEG 400 (A), Propylene glycol (B), *N/A= not available

The desirability function approach was applied in the present study using Design-Expert version 10.0.3 software, before optimization was done, the constraints (or goals) were set for all the responses. The goal for pH was set to be in range as the observed pH of all formulation were in the range of normal skin pH. The goal for viscosity and adhesivity was set to be maximized because all of the formulations have low viscosity, but the adhesivity was varied and gel with good adhesion is desirable in transdermal delivery to prolong the contact of formulation on the skin which allows the penetration of drug from the vehicle into the skin (Binder et al., 2019). Meanwhile, the goal for spreadability was set to minimize because the spreading diameter of 6 out of 8 formulas has already beyond the upper limit of the spreading diameter of the ideal semisolid formulation, whereas the other 2 formulations were inside the range. Therefore, to achieve gel with the ease of application but able to maintain longer contact with skin, the goal of spreadability was set to be minimized. Equal weight (1) and importance of 5 were given to all responses aside from pH whose importance was set to be 3. From these criteria, Design-Expert software gave 2 solutions with the desirability of 0.820 and 0.233 respectively following numerical optimization. Solution or formula with higher desirability value was chosen as an optimized formulation which consists of 75% PEG 400 and 25% PG or 47.25% b/b of PEG 400 and 15.75% b/b of PG in actual value which is similar to the composition of run 7. The desirability value of each solution was calculated by combining all the responses model and contour plot in one measurement. Best formulation with desirability value closer to 1, fulfilling the maximum requirement of response variable selected (Yadav et al., 2020). The optimized formulation then was subjected to the same physical characterization assay with the addition of stability studies and in vitro permeation studies. Confirmation of model used in optimization process was done by comparing the observed data and prediction value of each

parameter in the post-analysis confirmation menu of Design-Expert software. Results of model verification and accelerated stability study were depicted in Table 4.

Table 4. Model verification and physical properties of optimal Curcuma Longa Linn. Oleoresin non-aqueous gel formulation before and after accelerated stability testing

Observed parameters	Model confirmation			Before stability assay*	After stability assay	p-value (paired t-test)
	Prediction value	95% PI low	95% PI high			
Viscosity (d.pas)	6.698	5.93	7.47	6.44±0.19	6.16±0.16	0.032**
pH	5.00	4.71	5.29	5.16±0.09	4.96±0.06	0.101
Adhesivity (seconds)	6.640	5.19	8.09	6.05±0.11	5.97±0.09	0.117
Spreadability (cm)	6.615	5.90	7.33	6.94±0.06	6.86±0.09	0.118

*results expressed in the mean of n=3±standard deviation (SD), ** ($p<0.05$)

Three confirmation runs or replications were used to confirm that the model can predict the actual outcomes at the optimal setting determined from the analysis. The average observation data (data before stability assay) is compared to the prediction interval (both 95% PI low and high). Table 4 showed that the observation data from confirmation runs of all four parameters were within the confirmation 95% prediction interval, where PI is point prediction indicating that the model can accurately predict the value of all parameters of the optimal formula (Akala et al., 2015). Based on Table 4, the optimal formula resulted in non-aqueous gel with good characteristics such as pH of the formulation was within skin physiological pH value, can easily be spread on the skin due to high spreading diameter and low viscosity but also has good adhesion to the skin.

Stability is a term to describe the product's capability to retain its character within the specification during its storage period (Bajaj et al., 2021). Even though the NAG viscosity alteration was significantly different ($p < 0.05$), only a slight change in pH, adhesivity, and spreadability were observed after the NAG were subjected to 6 cycles of freeze-thaw stability test ($p > 0.05$). This result indicates the optimum formulation of *Curcuma longa* Linn. Oleoresin non-aqueous gel was quite stable.

In vitro diffusion study

Skin is known to be the main obstacle in transporting drugs via the transdermal route. One way to measure the drug's ability to pass the skin is by *in vitro* diffusion study. The study was performed using Franz diffusion cell for about 2 hours to the optimum NAG. The result of the study is shown in Figure 3.

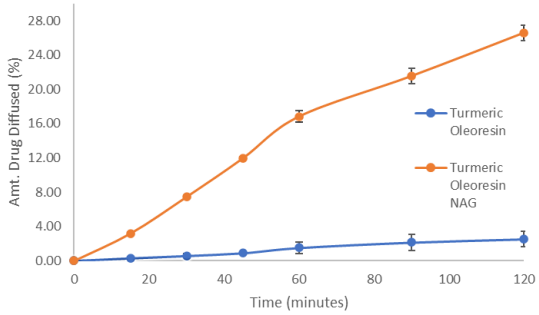

Figure 3. The diffusion profile of turmeric oleoresin and the non-aqueous gel optimum formula through shed snakeskin for 2 hours in PBS pH 7.4 (n=3)

The results showed that formulation was affected the *in vitro* transport of curcumin through the membrane. The optimum NAG can transport curcumin around 10 folds higher compare to the oleoresin without formulation. Around 26.58% curcumin were diffused from the NAG formula through the membrane (flux = 0.0458 mg.cm^{-2}). Meanwhile, only 2.52% of curcumin can pass the membrane without getting help from other ingredients (flux = 0.0043 mg.cm^{-2}). High lipophilicity (log P = 3.6) of curcumin could make the active ingredients detained in the skin surface before passing into the receptor medium. On the other hand, the presence of permeation enhancers (non-ionic surfactants) is suspected to be the reason behind the improvement of curcumin diffusion ability. Many studies reported that some chemicals (fatty oils and surfactants) could perturb stratum corneum lipids, hence allowing the drugs to pass across the layer. The permeability characteristics of lipid bilayers are amplified by the reduction of lipid bilayers' crystallinity due to the seep of non-ionic surfactants into the intercellular lipid bilayers. Another research said that the non-ionic surfactants can increase the drug's thermodynamic activity by causing sebum emulsification (Akhtar et al., 2011). The membrane's permeability amplification hence causing the improvement of the flux of curcumin through the skin. The study shows that the preparation of *Curcuma Longa* Linn. oleoresin into the non-aqueous gel is promising to be further developed as the alternative delivery system on RA treatment.

CONCLUSION

Based on the research it can be concluded that the *Curcuma longa* Linn. oleoresin can be formulated into a non-aqueous gel system, which shows fair gel physical characteristics with good stability and good ability to permeate across the skin membrane. The study shows that the preparation of *Curcuma Longa* Linn. oleoresin into the non-aqueous gel is promising to be further developed as the alternative delivery system on RA treatment.

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