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RESEARCH ARTICLE

Development of novel curcumin nanoemulgel: Optimisation, characterisation, and *ex vivo* permeation

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Abstract

Introduction: Curcumin (Crc) is widely used as an antioxidant and an anti-inflammatory agent. Its low solubility limits its oral bioavailability, thus the need to develop a transdermal nanoformulation of Crc. **Aim:** This study aimed to obtain the stable formula of Crc-loaded nanoemulgels (Crc-NEGs) possessing good characteristics. **Methods:** The nanoemulsion (NE) was prepared by titration of the water phase into the mixture of oil, surfactant, and cosurfactant. Crc-NEs optimum formula was obtained by the simplex lattice design (SLD) method. Crc-NEGs were prepared using Carbopol 940 as the gelling agent, and subsequently, its freeze-thaw stability was observed. The *ex vivo* permeation study of Crc-NEGs was conducted using Franz diffusion cell. **Results:** The optimum formula of Crc-NEs showed good characteristics in terms of transmittance, particle size, polydispersity index, and zeta potential. Crc-NEGs were found stable through freeze-thaw stability. The *ex vivo* permeation study illustrated the higher amount of Crc penetrated from NEGs compared to the control *(p* <0,05). **Conclusion:** The Crc-NEGs formula has the potential to be the novel effective delivery method of curcumin.

Introduction

Curcumin (Crc) is a compound isolated from the rhizome of *Curcuma longa* Linn possessing antioxidant, anti-inflammatory, antimicrobial, and anticancer properties (Tønnesen & Karlsen, 1985; Rachmawati *et al.,* 2015; Hewlings & Kalman, 2017). However, curcumin's low water solubility becomes a significant drawback, as this leads to a decrease in bioavailability and lower its efficacy when administered orally (Rachmawati *et al.,* 2015). Hence, to overcome this problem, transdermal delivery is suggested. This route is expected to be a promising solution.

Nanoemulsions (NEs) are one of the beneficial dosage forms for transdermal delivery. Possessing the dispersed particle size of lower than 200 nanometers (nm), NEs are visually clear and thermodynamically stable (Tang *et al.,* 2012; Lala & Awari, 2014). Moreover, the nano-scale particles increase the penetration ability into the deeper skin layers (Su *et al.,* 2017).

A suitable optimisation method is needed to obtain the optimum formula of Crc-loaded NEs (CrcNEs) with sufficient skin penetration. Simplex lattice design (SLD) is a statistical-based method that could be utilised to describe the relations between CrcNEs constituents (e.g., surfactants-cosurfactants mixtures (Smix), oil, and aqueous phase). This method outweighs trial and error, which is an ineffective and time-consuming method (Duangjit *et al.,* 2014).

The transdermal delivery of NEs can be adequately conducted by incorporating these NEs into hydrogel bases to form nanoemulgels (NEGs). Increased adhesion ability generated by a gel system will localise drug penetration and hinder active ingredients from being washed by sweat on the skin surface (Ahmad *et al.,* 2019; Muhtadi *et al.,* 2020).

This study aims to develop an effective transdermal delivery of Crc by incorporating it into a nano vehicle. The specific purposes of this study are to observe the characteristics of the nanoformulation and assess its skin penetration ability through *ex vivo* diffusion. To the best of our knowledge, no studies have yet formulated the nano dosage form of Crc through computational optimisation.

Materials and methods

Materials

Curcumin (Crc) (Merck, Germany); virgin coconut oil (VCO) (PT. Inhil Sarimas Kelapa, Indonesia); tween 80 (TW80) (Kao, Indonesia); transcutol P (TP) (Gattefosse, France); carbopol 940 (C940) (STIFAR Riau, Indonesia); sodium metabisulfite (Zhejiang Medicine, China); ethanol, sodium hydroxide, potassium dihydrogen phosphate (Merck, USA).

Solubility study

An excess of Crc was put into four tubes consisting of 5 ml of VCO, TW80, TP, and water, respectively, then stirred for 24 hours and centrifuged at 3000 rpm for 15 minutes. The supernatants were analysed using UV-Vis Spectrophotometer (Shimadzu UV-1800) at 428 nm.

Construction of pseudo-ternary phase diagram and Formula optimisation using SLD

TW80:TP (Smix) were mixed in several ratios, VCO was mixed with Smix(s), yielding VCO:Smix(TW80:TP) ratios (Table I), and added with water. Visual behaviour was plotted into the phase diagram (ProSim Ternary Diagram 1.0). CrcNEs' optimisation was conducted using Design-Expert (Stat-Ease Inc., USA). The factors were concentrations of water (X_1), VCO (X_2), and Smix (X_3), and the response was transmittance (Y_1) (Duangjit *et al.,* 2014).

Table I: The visual appearance at each additional point of the aqueous phase

The Ratio of oil:Smix (TW80:TP) (ml)	Observed appearance following the addition of aqueous phase																	
	0 µl	100 µl	100 µl	100 µl	100 µl	100 µl	100 µl	150 µl	200 µl	200 µl	300 µl	0.35 ml	0.5 ml	0.65 ml	1.0 ml	1.5 ml	3.5 ml	1.1 ml
1:9 (7:1)	E	E	E	E	E	E	E	E	E	E	E	E	E	E	NE	NE	NE	NE
1:9 (8:1)	NE	E	E	E	E	E	E	E	E	E	E	E	E	E	NE	NE	NE	NE
1:9 (9:1)	NE	E	E	E	E	E	E	E	E	E	E	E	E	E	NE	NE	NE	NE
1:7 (7:1)	NE	E	E	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE
1:7 (8:1)	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	E	E	E	E	NE	NE	NE	NE
1:7 (9:1)	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	E	E	E	NE	NE	NE	NE
1:8 (7:1)	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE
1:8 (8:1)	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	E	E	E	NE	NE	NE
1:8 (9:1)	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	E	E	E	NE	NE	NE	NE

NE- Nanoemulsions; E- Emulsions

Preparation, transmittance, and the prediction and verification of Curcumin-loaded nanoemulsion (CrcNES)

Crc (0.25% in the final formula) was added to VCO and stirred at 300 rpm. TW80, TP, and water were sequentially added into the mixture (Duangjit *et al.,* 2014; Muhtadi *et al.,* 2019). The transmittance of CrcNEs was analysed using a UV-Vis spectrophotometer (Shimadzu UV-1800) at 650 nm with water as the blank (Syukri *et al.,* 2018). The prediction of the optimum CrcNEs was examined by assessing several relevant values of Y_1 in Design-Expert software. Moreover, the verification of the optimum condition was assessed by a one-sample t-test with a confidence interval of 95% (p>0.05) (Shiyan *et al.,* 2018).

Particle size, polydispersity index, and zeta potential

Studies were carried out using a particle size analyser (Horiba SZ-100). One millilitre of the sample was put into a flask and was diluted with 25 ml of distilled water and analysed in triplicate.

Preparation of Curcumin-loaded nanoemulgel Crc-NEGs and freeze-thaw stability study

C940 (0.4 g) was sprinkled on the surface of Crc-NE and stirred (300 rpm) at a warm temperature. Moreover, sodium metabisulfite was added and stirred until the homogenous gel was formed (Muhtadi *et al.,* 2020). Furthermore, CrcNEGs were stored at 4°C for 48 hours and 40°C for 48 hours (1 cycle), with a total of 6 cycles. The phase separation, pH (Ohaus ST300), viscosity (Brookfield DV-1 Ametek), and spreadability were observed (Donsì *et al.,* 2011).

Ex vivo skin permeation study

The dorsal skin hair of Wistar male mice was trimmed and cut to a circular shape. The excised skin was then immersed in phosphate buffer (PB) pH 7.4 for 30 minutes. CrcNEGs (5 grams) were placed on the donor compartment of the Franz diffusion cell, and the receptor solution (PB pH 7.4) was stirred at 37±0.5°C and 100 rpm. Aliquots (5 ml) were collected in the span of 8 hours and were measured using a UV-Vis spectrophotometer (Shimadzu UV-1800) at 430 nm. Cumulative Crc permeated (µg/cm^2), flux (µg/cm^2.h), and permeability coefficient (cm/h) was calculated from the detected Crc concentration (Kim *et al.,* 2008).

Statistical analysis

The results were provided as mean±standard deviation. The effects of factors against response in the SLD were analysed using ANOVA. The optimisation study was verified and analysed using a one-sample *t*-test. *Ex vivo* permeation results were analysed using a two-sample *t*-test.

Results

Solubility study of Crc

Solubility values of Crc in VCO, TW80, TP, and water were 1.65 ± 140.31 mg/ml, 132,51 ± 3.60 mg/ml, 59.28 ± 11.85 mg/ml, and 0.59 µg/ml, respectively.

Pseudo-ternary phase diagram

The VCO:Smix(TW80:TP) ratio of 1:8(7:1) yielded a transparent visuality and was used further in the SLD. Figure 1 illustrates the pseudo-ternary phase diagram.

Formula optimisation using SLD

The upper and lower limits of X_1, X_2, and X_3 concentrations were 62-66%, 0-4%, and 30-34%, respectively. The SLD runs are presented in Table II with the Y_1 range of 51.6-93.2%. The several statistical indicators of the SLD are presented in Table II.

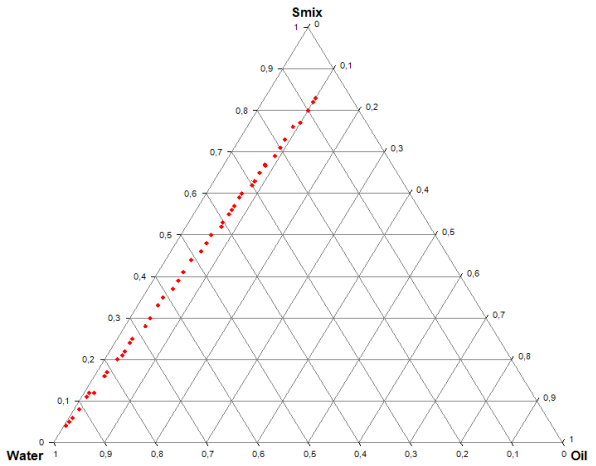


Figure 1: The pseudo-ternary phase diagram indicating the area of NEs formation, which was marked by red dots

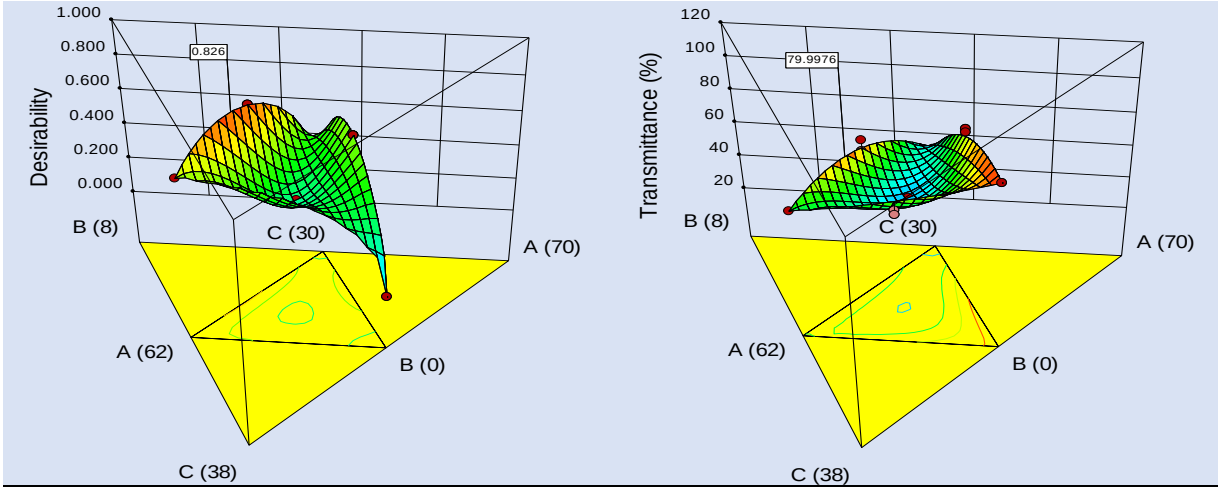
Table II: Design of optimisation of CrcNEs using SLD and the statistical parameters of the response of transmittance Y_1

Number of runs	Water (%) (X_1)	VCO (%) (X_2)	Smix (%) (X_3)	Transmittance (%) (Y_1)
1	64	4	32	77.1
2	64	2	34	70
3	66	0	34	92.2
4	64	4	32	76.3
5	62	4	34	68.9
6	64,67	2.67	32.67	60.1
7	64	4	32	83.6
8	64	2	34	72.7
9	66	2	32	93.2
10	64	2	34	91
11	66	2	32	81.8
12	66	4	30	51.6
13	66	2	32	91.2

Parameters	Values
Model p-value	0.045
Deviation standard	7.82
Mean	77.67%
CV	10.07%
R^2	0.8179
Adequate precision	7.072
Regression equation	$Y_1 = 764.63\ X_1 + 733.37\ X_2 + 643.37\ X_3 - 2789.60\ X_1\ X_2 - 2447.20\ X_1\ X_3 - 2477.87\ X_2\ X_3 + 5494.40\ X_1\ X_2\ X_3$

The prediction and verification

The selected concentrations were 4.00% (VCO), 32.47% (Smix(7:1)), and 63.53% (water). Figure 2 presents desirability (0.826) and Y_1 prediction (79.99%), indicating no difference between the predicted and observed Y_1 ($p > 0.05$).



(a) (b)

Figure 2: The 3D-response surface plot of desirability (a) and prediction of transmittance value (b)

Particle size, polydispersity index, and zeta potential

The results showed the particle size of lower than 200 nm, polydispersity index of below 0.7, and the zeta potential of higher than -20 mV (Table III).

Table III: Results of CrcNEs characterisation (n = 3)

Parameters	Values
Particle size	12.73 ± 0.81 nm
Polydispersity index	0.325 ± 0.02
Zeta potential	-26.6 ± 0.44 mV

Freeze-thaw stability of CrcNEGs

The results indicated stable CrcNEGs through freeze-thaw stability based on several parameters, such as phase separation, pH, viscosity, and spreadability (Table IV).

Table IV: Results of freeze-thaw stability of CrcNEGs

Parameters	Number of cycles					
	1	2	3	4	5	6
Phase separation	NP	NP	NP	NP	NP	NP
pH	4.55	4.58	4.55	4.89	4.95	5.05
Viscosity (cP)	340 ± 34.64	NM	NM	NM	NM	346.67 ± 42.63
Spreadability (cm^2)	188.59	186.17	188.59	188.59	186.17	186.17

NP = No phase separation; NM = Not measured

Ex vivo skin permeation

The cumulative Crc permeated for eight hours from the NEGs (25.16±14.20 µg/cm^2) was higher than the control (16.57±3.54 µg/cm^2) (Figure 3). The flux of CrcNEGs (2.46±2.19 µg/cm^2.h) was higher compared to the plain Crc (1.92±0.56 µg/cm^2.h), with $p > 0.05$.

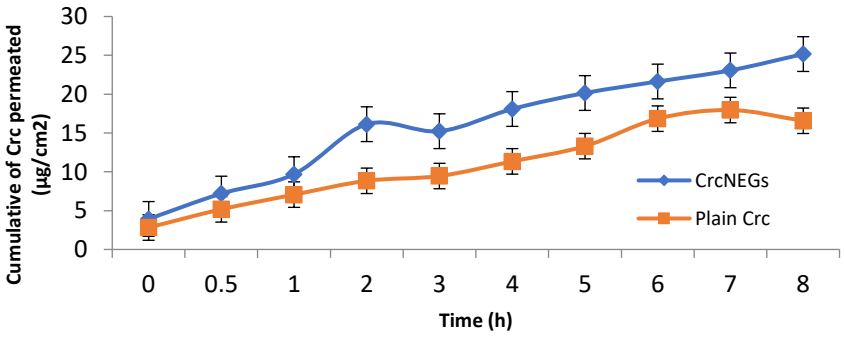


Figure 3: Cumulative of Crc permeated (n = 3)

Discussion

The solubility study illustrated that Crc was loaded into the oil droplets, as the manufactured NEs were in the state of oil in water (O/W). Preliminary studies had shown equilibrium points that produced a transparent NE from the initial until the last drops of the water

phase (Shafiq-un-Nabi *et al.,* 2007). In this study, transparent NEs were produced in each water-dropping point. VCO:Smix(TW80:TP) of 1:8(7:1) was selected for further optimisation as it had lower surfactant compared to the VCO:Smix ratio of 1:9 because the higher concentration of surfactants potentially ignites skin irritation (Effendy & Maibach, 1995).

The used factors had significant effects on the NEs' transmittance. It was clear that each factor had a positive effect or merely increased the transmittance values. Conversely, the absence of one of the factors decreased the transmittance of CrcNEs. The value of R^2 of more than 0.7, the adequate precision of higher than 4.0, indicated that the model used for the optimisation of CrcNEs described the correlation of factors and responses (Setyawan *et al.,* 2018; Shiyan *et al.,* 2018).

The desirability value (close to 1) indicated the model's ability to accurately predict the actual transmittance of the optimum formula (Candioti *et al.,* 2014). Transmittance reflects the clarity of NEs favoured with values closer to 100% (Laxmi *et al.,* 2015). The formula's visual appearance was not very clear though transmittances were over 80%. However, the further particle size study showed that the obtained transmittance was acceptable.

The particle size, polydispersity index, and zeta potential significantly affected the NE behaviour. Moreover, the particle size also determines NE penetration ability, where sizes lower than 50 nm could penetrate deeply through the skin (Lala & Awari, 2014; Cai *et al.,* 2016). The polydispersity index (PI) figures the degree of uniformity of the dispersed particles. A PI lower than 0.7 is preferred, as it indicates a narrow distribution of particles. PIs close to 1.0 potentially suggest Ostwald ripening, the phenomenon when smaller particles irreversibly merge with larger ones, leading to coalescence (Mason *et al.,* 2006). Zeta potential illustrates the surface charge of particles, which forms the repulsion force between each globule. More positive or negative zeta potential hinders the integration of particles. The results of this study showed zeta potential higher than -20 mV, considered good. The negative charge of CrcNEs was probably due to the presence of glycols in both TW80 and TP and the existence of the VCO's anionic groups (Laxmi *et al.,* 2015).

The freeze-thaw stability of CrcNEGs indicated no signs of an unstable formulation. The phase separation study result illustrates the endurance of CrcNEGs against temperature changes. The pH of CrcNEGs through six cycles indicated its suitability to be applied on the skin surface since the pH(s) were in the normal range of the skin pH. The obtained viscosity and spreadability values showed that CrcNEGs could be easily applied to the skin (Ueda *et al.,* 2010).

In the *ex vivo* permeation study, the presence of TW80 aided the drug penetration through the skin membrane because TW80 plays the role of a penetration enhancer, extracting the stratum corneum lipids and subsequently plunging the stratum corneum barrier ability (Khurana *et al.,* 2013). Further study is needed to compare the penetration of CrcNEGs with Crc conventional gels so that the difference can be assessed.

Conclusion

The development of CrcNEGs was successfully conducted, with good characteristics of NEs and a stable form of NEGs. Furthermore, NEGs had sufficient penetration ability. To sum up, CrcNEGs can be the novel effective delivery method of curcumin.

Conflict of interest

There is no conflict of interest associated with the present study.

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