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Development of Cinchonine Emulgel as a Topical Antibacterial using Response Surface Methodology

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ABSTRACT

Cinchonine has potential as a topical antibiotic with a mechanism of action that inhibits bacterial cell wall formation. Cinchonine is insoluble in water, resulting in poor penetration. The aim of the research was to develop a cinchonine emulgel formula using the Response Surface Method (D-optimal) as an antibacterial with parameters spreadability, adhesiveness, pH, and viscosity. The optimum cinchonine emulgel was determined based on the desirability value (range 0-1). The release test of cinchonine emulgel was carried out using a 12-14 kD cut-off dialysis bag. Cinchonine emulgel was tested for antibacterial activity against Pseudomonas aeruginosa and Staphylococcus aureus by the diffusion method with a positive control and a negative control. The inhibition zone parameters were carried out with observation intervals of 18 and 24 hours. Stability test of the optimum formula was carried out by cycling test and accelerated method with test parameters including spreadability, adhesiveness, and pH. The optimum composition of cinchonine emulgel consisted of cinchonine 1.5; HPMC 2; glycerin 1; tween 80 4.99; propylene glycol 1.14; oleic acid 14.84; and deionized water up to 100% with a desirability value: 0.504, spreadability: 3.92±0.38cm; adhesiveness: 2.17±0.29 second, pH: 4.72±0.11; and viscosity: 12050±900.39cP ($p<0.05$). The release of cinchonine emulgel was 19.25% controlled. Observation of inhibition zone at 18 and 24 hours respectively for cinchonine emulgel preparation against Pseudomonas aeruginosa was 7.68±0.14 and 6.50±0.37mm, Staphylococcus aureus was 10.47±0.37 and 10.36±0.69mm compared to control (gentamicin ointment: 18.15±0.90, and 16.99±1.61mm; blank control: 0.00±0.00, and 0.00±0.00mm). Cinchonine emulgel had good stability during short-term storage.

***Keywords*:** Antibacterial; Cinchonine; D-optimal; Emulgel; Response Surface Methodology

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INTRODUCTION

Cinchonine (CN) is one of the active ingredients derived from the fractionation of Cinchona extract. CN has antibacterial activity against Pseudomonas aeruginosa (P. Aeruginosa) with an inhibition zone diameter of 19.2 mm (Minimum Inhibitory Concentration (MIC): 12.5 µg/mL), Staphylococcus aureus (S. Aureus) with an inhibition zone diameter of 13.2 mm (MIC: 50 µg/mL)[1] and Cutibacterium acnes (C. acne) with an inhibition zone diameter of 13.26 mm.[2] The antibacterial activity of CN is thought to be due to a mechanism of bacterial cell membrane disruption. CN interacts with bacterial cell membrane phospholipids, causing changes in permeability, leakage, and lysis of the bacterial cell wall.[3,4] CN has fewer side effects compared to other Cinchona alkaloids (Quinine, Quinidine, and Cinchonidine).[5,6] Therefore, CN has the potential to be a candidate antibiotic for topical use.[2] However, the characteristics of CN which is practically insoluble in water causes low permeability of CN to the target organ (skin in the epidermis and dermis layers).[2]

Dosage form plays a crucial role in enhancing the activity, stability, and safety of a product. CN in gel form with a concentration of 1.5% is able to inhibit bacterial growth of 7.75 ± 0.22 mm (observation at 18 hours) and 5.93 ± 0.38 mm (observation at 24 hours).[2] The gel dosage form is thought to be suboptimal in facilitating CN which has high hydrophobicity. Emulgel is a dosage form that can facilitate the transport of hydrophilic and lipophilic active ingredients. This is one of the advantages of emulgels, which are expected to overcome the skin barrier, increasing the activity, stability, and safety of the product.[6] The concentration of additives (lipids and gelling agents) used in the formulation affects the release of CN from the emulgel preparation. Therefore, the concentration of each additive used in the formulation needs to be optimized to obtain the optimum emulgel formula.[2]

Design-expert software is widely used in formula development because it has proven to be effective and efficient in obtaining the optimum dosage formula.[2,7] This study aims to develop and obtain the optimum formula of CN-emulgel using Response Surface Methodology (RSM) as an antibacterial against P. Aeruginosa and S. Aureus.

METHODS

Materials and Equipment

CN (PT. SIL, Indonesia), hydroxypropyl methylcellulose (HPMC), glycerin, oleic acid, tween 80, propylene glycol, oleic acid, DMDM hydantoin, butylated hydroxytoluene, alcohol (Bratachem, Bandung, Indonesia), deionized water (Amidis, Semarang, Indonesia), hydrochloric acid (HCl), sodium hydroxide (NaOH), sodium chloride (NaCl), Phosphate buffered saline (PBS) pH 7.4 (Merck, Germany), Mueller Hinton Agar (MHA) media (Oxoid, England), P. aeruginosa (ATCC27853) (Laboratorium of the University of Indonesia, Indonesia), and S. aureus (ATCC25923) (Microbiology Laboratory of the University of Muhammadiyah Purwokerto, Indonesia), gentamicin ointment 0,1%, and commonly used glassware in the laboratory.

Magnetic stirrer (RT 15, IKA, Germany), pH meter (starter 3100, Ohaus, USA), viscometer (Brookfield dv2t, Ametek, USA), vortex (Stuart scientific SA5, Monotaro, Japan), UV-Vis spectrophotometer (UV-2600i, Shimadzu, Japan), dialysis bag (Sigma, Europe), autoclave (Hiclave HVP-50, Hirayama, Japan), incubator (Memmert, Germany), micropipette (Eppendorf, Germany), caliper (Doziro, Japan), LAF (Massotte, Indonesia), analytical balance (Ohaus, USA), hot plate (C-Mag Hs 7, IKA, Germany), pH meter (starter 3100, Ohaus Japan), climatic chamber (Memmert, Germany), refrigerator (Toshiba, Japan) and laboratory glassware.

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Animals

Male rabbit of the New Zealand strain of 4, weighing about 2 kg and aged around 6-7 months (rabbit purwokerto, Indonesia). Ethics of dermal irritation test were obtained from KEPK/UMP/76/VI/2025.

Validation of CN analysis method with UV- Visible spectrophotometry

Confirmation of the maximum wavelength (λmax) of CN

The CN solution was prepared at a concentration of 50 mg/100 mL (500 ppm). Two mL of the CN solution was analyzed in the wavelength range of 250–350 nm at 25°C. Acceptance parameters: The maximum wavelength (λmax) of CN was 288–290 nm.[8]

Determination of linearity

CN solution was prepared in 7 concentration variations of 20, 30, 40, 50, 60, 70, and 80 ppm. Samples were analyzed at λmax CN, temperature 25°C. The response/absorbance obtained from each sample was recorded and a relationship curve was created between concentration (x) and response (y) following the equation y = bx + a. The acceptance parameter of the linearity of the analysis method is the slope value (b) which is close to 1.[8]

Determining the Limit of Detection (LoD)

The LoD of the analysis method is determined by following the following equation: [8]

$$LoD = (3.SD/b)$$

SD : Standard Deviation
B : Slope of the equation Y=a+bx

Determining the Limit of Quantification (LoQ)

The LoQ of the analysis method is determined by following the following equation: [8]

$$LoQ = (10.SD/b)$$

SD : Standard Deviation
B : Slope of the equation Y=a+bx

Determination of intraday accuracy and precision

The accuracy of the analytical method was determined using the placebo spike method. Samples were prepared by making a physical mixture of CN:blank emulgel (30:70) with varying concentrations of 30, 60, and 70 ppm (6 replications). 0.5 mL of the CN-blank mixture was dissolved in 3 mL of chloroform, added with 8 mL of HCl, and vortexed for 5 minutes. The aqueous phase was analyzed using a UV-Vis spectrophotometer at λmax CN, temperature 25°C. Accuracy was calculated using the following equation: [8]

$$\text{Percentage recovery} = (Ca/Ct) \times 100$$

Ca: CN concentration obtained from the analysis results
Ct: Theoretical concentration of CN added to the blank (emulgel)

Precision is determined from the standard deviation (SD) or relative standard deviation (RSD). Precision is expressed as repeatability or reproducibility with an acceptance parameter of <2%.[8]

Optimization of CN-emulgel

CN-Emulgel formula optimization was carried out using Design Expert software version 13 with the Response surface methodology (RSM) - D-Optimal method.

Table 1. CN-emulgel formula

Ingredients	Function	Concentration (%)
Gel phase:		
HPMC	Gelling agent	0.5-2
Glycerin	Humectant	1-30
DMDM hydantoin	Preservative	0.5
Emulsion phase:		
CN	Active ingredient	1.5
Oleic acid	Liquid lipids	1-5
BHT	Antioxidant	1.5
Tween 80	Emulsifier	1-15
Propilen glycol	Penetration enhancer	1-15
deionized water	Solvent	Ad 100

The optimization of the CN-emulgel formulation was designed using Design Expert version 13 software using the response surface d-optimal method. D-optimal was used to facilitate the identification of interactions that could occur due to the influence of various ingredients on research results and to

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obtain the appropriate formula. The optimum formula was determined based on the desirability value. Desirability is the formula's proximity to the established final quality specifications.[7] The results of running using the design expert software version 13 are shown in Table 2 with observation parameters including: response model, lack-of-fit, R2, adjusted R2 and desirability value.

Preparation of CN-Emulgel

Gel: HPMC was developed using deionized water (70±2°C), homogenized using a magnetic stirrer (speed 300±20 rpm) until a gel mass was formed, then glycerin and DMDM hydantoin were added and homogenized using a magnetic stirrer (mass 1).[2]

CN-Emulsion: The oil phase (CN, oleic acid and BHT), and the water phase (tween 80, propylene glycol and aqua deion) were each heated to 70±2°C and homogenized using a magnetic stirrer (speed 300±20 rpm). Then the water phase was added to the oil phase, homogenized using a magnetic stirrer (speed 300±20 rpm) (mass 2).[9]

CN-emulgel: Mass 2 was added to mass 1 and the mixture was homogenized using a magnetic stirrer (speed 300±20 rpm) for 10 minutes.[10,11]

Table 2. Formula Design Using Design Expert - RSM

Run	HPMC (% w/w)	Glycerin (%w/w)	Tween 80 (%w/w)	Propylene Glycol (%w/w)	Oleic Acid (%w/w)
1	1.22	13.47	8.91	1	3.351
2	1.299	1	15	15	1
3	0.5	30	3.8	15	5
4	0.575	30	15	3.52	5
5	0.5	9.7	15	15	5
6	1.925	1	1	1	4.6
7	1.241	14.92	1	8.56	3.52
8	0.5	11.875	1.28	15	1
9	0.5	10.86	15	2.095	1
10	1.241	14.96	1	8.56	3.52
11	2	26.085	3.1	2.4	5
12	0.5	30	1	1	2
13	0.5	1	1	1	5
14	0.613	1	7.72	8.63	2.74
15	2	14.485	10,8	11.08	2.02
16	0.5	30	15	15	2.32
17	1.22	13.47	8.91	1	3.351
18	2	30	15	1	1.7
19	2	30	1	15	1.92
20	1.895	1	3.8	15	4.8
21	1.25	27.535	7.65	8	1
22	1.663	30	15	15	5
23	2	14.485	10.8	11.08	2.02
24	2	30	1	1	1.623
25	1.783	1	1	15	1
26	1.925	1	15	4.15	4.68
27	2	1	1	1	1

Confirmation of optimum CN-emulgel dosage form

Three replicates of the optimum CN-emulgel were made, obtained from the DoE recommendation of 100 g according to the CN-emulgel manufacturing procedure. The CN-emulgel was characterized by the following evaluations:

Spreadability test

CN-emulgel (0.5 g) was applied to a graduated watch glass and then covered with another watch glass. Spreadability was determined by measuring the emulgel diameter after 1 minute under no-load conditions, with loads of 50, 100, and 150 g. The acceptable parameter for emulgel spreadability was a spreadability with a diameter of 5-7 cm.[2]

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Adhesion test

CN-emulgel (0.5 g)was applied to a glass slide, then covered with another glass slide, and a 1 kg load was applied for 1 minute. The adhesiveness acceptability parameter was determined by the time required for the two glass slides to separate by >1 second.[10]

pH determination

CN-emulgel (1 g) was dissolved in deionized water and homogenized by vortexing for 3 minutes. The pH of the CN-emulgel was determined at 25°C. The acceptable pH range for the emulgel is 4.5-6.5.[11]

CN-emulgel release test

The CN-emulgel release test was conducted in vitro using a dialysis bag with a cut-off of 12-14 kD. CN-emulgel (1 g) was placed into the dialysis bag. The sample (CN-emulgel in the dialysis bag) was placed in PBS (50 mL, pH 7.4) with a magnetic stirrer stirring speed of 50±10 rpm, a temperature of 37±2^{o}C. The percentage of CN release was determined at time intervals of 5, 10, 15, 30, 45, 60, 90, 120, 150, and 180 minutes. 2 mL of the sample (release medium: PBS) was taken and analyzed using a UV-Vis spectrophotometer at λmax CN, a temperature of 25^{o}C. After sampling, the sample was replaced with a new PBS with the same amount.[8]

Antibacterial activity test

The activity test was conducted using the diffusion method (wells) with 3 test groups: blank control (emulgel base), comparison control (gentamicin ointment 0.1%) and test sample (CN-emulgel 1.5%). The media mixture (MHA 12 mL and 0.1 mL of bacterial suspension (P. Aeruginosa, and S. Aureus), holes are made with a certain diameter (6 mm) in the medium. Samples of 0.1 g were placed into each well, respectively, for the blank control group, the comparison control group, and the test sample, and incubated at 37°C for 24 hours. Observations of the inhibition zone were carried out at 18 and 24 hour intervals, and the diameter of the inhibition zone was measured using a vernier caliper. [12]

Cycling test and accelerated stability test

The cycling stability test was conducted on samples stored for six cycles. Each cycle lasted 48 hours (24 hours at 4°C and 24 hours at 40°C). Observations were made at cycle intervals of 1, 2, 3, 4, 5, and 6, with parameters such as homogeneity, organoleptic properties, pH, adhesion, and spreadability.[13]

Accelerated stability tests were conducted at storage conditions of 40 ± 2^{o}C and 75% ± 5% relative humidity (RH) for 28 days with observation intervals of 0, 7, 14, 21, and 28 days. Characteristics observed during the stability test included: organoleptic (color, odor, and texture), homogeneity, pH, adhesiveness, and spreadability.[14]

Skin irritation test

The test animals were acclimatized for 7 days and placed in individual cages. The backs of the test animals were shaved 24 hours before the irritation test, covering an area of approximately 6(2x3)cm^{2}. Shaving began from the shoulder blade to the hip bone.[15] The CN-emulgel skin irritation test has been approved by Komite Etik Penelitian Kesehatan Universitas Muhammadiyah Purwokerto (KEPK-UMP) No. KEPK/UMP/76/VI/2025.

Statistical analysis

Data are presented as mean ± SD. The optimum formula (n = 3) was determined based on the desirability value (~1). Desirability is the value of the formula's proximity to the established final quality specifications. The confirmation and validation process for the optimum CN-emulgel was also carried out using One-Way ANOVA and Statistical Product and Service Solutions (SPPS) software with the One-Sample T-test method ($p<0.05$).

RESULTS AND DISCUSSION

The maximum wavelength (λmax) of CN is determined to obtain an analysis

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condition that produces the highest response (absorbance) as shown in Figure 1. The maximum wavelength is important to determine because it can affect the analysis results. Using the right maximum wavelength can produce analysis data with high accuracy, precision, and selectivity.[16] The analysis results showed that the maximum wavelength of CN was 289 nm. This maximum wavelength was confirmed by previous studies and proved that CN was not contaminated or degraded.[8]

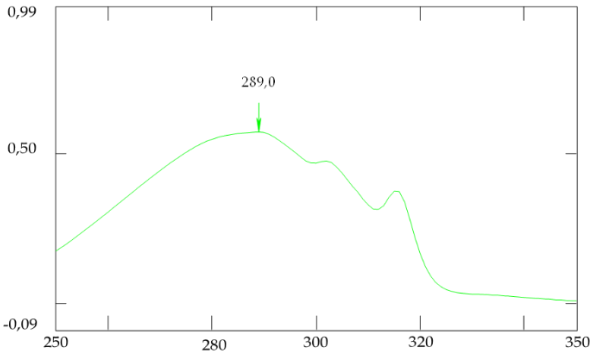

Figure 1. Confirmation of the maximum wavelength of CN

The data in Figure 2 shows the relationship between concentration and the response resulting from the analysis process using UV-Vis spectrophotometry. The data indicate that changes in CN concentration are directly proportional to the response. This is indicated by a linearity value (R2) of 0.9982 and a regression equation of y = 0.0083x + 0.0431. [8]

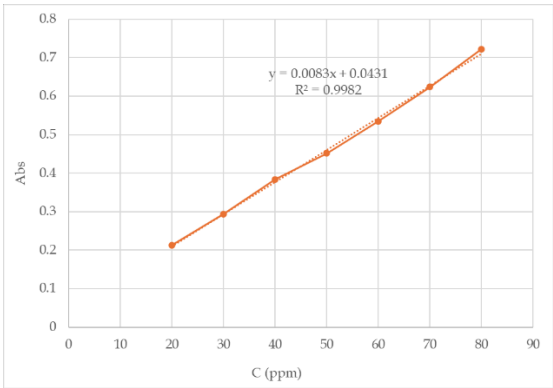

Figure 2. Relationship curve between concentration variation and response

The data presented in Table 3 shows the limit of detection (LoD) and limit of quantitation (LoQ) values for the analysis. The LoD value was 3,56 ppm, and the LoQ was 10,79 ppm. The LoD value indicates the minimum detectable concentration of CN, while the LoQ value is the minimum concentration that can be statistically quantified. These data indicate that the analytical method has high sensitivity in detecting CN.[8,16]

Table 3. LoD and LoQ values

C (ppm)	Abs (y)	C′ (ppm)	(y′)	(y-y′)	(y-y′)²
20	0.213	20.47	0.203	0.0096	9.21E-05
30	0.294	30.23	0.288	0.0056	3.13E-05
40	0.384	41.07	0.373	0.0106	0.00011
50	0.452	49.27	0.458	-0.0064	4.09E-05
60	0.535	59.27	0.543	-0.0084	7.05E-05
70	0.624	69.99	0.628	-0.0044	1.93E-05
80	0.722	81.80	0.713	0.0086	7.39E-05
Total					0.00044
S(y/x)²					8.8E-05
S(y/x)					0.0093
LOD (ppm)					3.56
LOQ (ppm)					10.79

The data in Table 4 shows that the analytical method has high accuracy and precision. High accuracy and precision are characterized by recovery values reaching 98–100%, with SD and RSD values <2%. The resulting precision values indicate that the analytical method has high reproducibility when used for repeated sample analysis.[8,16] From all the validation parameters of the method that have been determined, it shows that the CN analysis method using a UV-Vis spectrophotometer is compatible with high accuracy and precision.[8,17]

CN-Emulgel formula optimization was performed using RSM – D-optimal. Optimization was performed on HPMC, glycerin, Tween 80, propylene glycol, and oleic acid. Formula optimization was carried out with the aim of obtaining the optimum composition of each ingredient to produce a high-quality emulgel preparation. High-quality emulgel specifications were determined based on acceptance criteria from each quality evaluation conducted.[2]

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Table 4. Accuracy and precision values of the analysis methods

C (ppm)	Abs (y)	C'	Recovery (%)	Average (%)	SD	RSD
30	0.294	30.23	100.76	100.56	1.07	0.01
30	0.293	30.11	100.36			
30	0.290	29.75	99.16			
30	0.292	29.99	99.96			
30	0.298	30.72	102.37			
30	0.294	30.23	100.76			
60	0.535	59.27	98.78	98.88	0.58	0.01
60	0.534	59.14	98.57			
60	0.539	59.75	99.58			
60	0.536	59.39	98.98			
60	0.538	59.63	99.38			
60	0.531	58.78	97.97			
70	0.624	69.99	99.98	100.30	0.33	0.003
70	0.629	70.59	100.84			
70	0.626	70.23	100.33			
70	0.627	70.35	100.50			
70	0.625	70.11	100.15			
70	0.624	69.99	99.98			

Table 5 shows the resulting formula variations of 27 runs with evaluations including spreadability, adhesion, pH, and viscosity. The optimum CN-emulgel formula is determined from the desirability value. RSM – D-optimal provides an optimum formula recommendation with a composition of CN 1.5, HPMC 2, glycerin 1, tween 80 4.99, propylene glycol 1.14, oleic acid 14.84 and aqua deion ad 100% presented in Table 6. Characterization of the CN-emulgel preparation spreadability 3.92 ± 0.38 cm, adhesion 2.17 ± 0.29 second, pH 4.72 ± 0.11 and viscosity 12050 ± 900.39 cP.[2,18]

Observations on the response (spreadability, adhesiveness, pH and viscosity) of each variation of the concentration of the variables (HPMC, glycerin, tween 80, propylene glycol and olic acid) are presented in Table 5. The response model of spreadability shows a linear model (p: 0.22, Model F-value of 5.49, Lack of fit (p: 7.98), Lack of Fit F-value of 6.15, R^2: 0.5668, Adjusted R^2 0.4636). These data indicate that oleic acid is the most dominant excipient and influences the spreadability. Increasing the concentration of oleic acid will cause a decrease in the spreadability of the emulgel preparation. This is in line with the pharmaceutical concept which states that if the viscosity of the preparation increases it will cause a decrease in the spreadability.[18–20]

Table 5. Optimization of CN-emulgel with RSM – D-optimal

Run	Characterization			
	Spreadability test (cm)	Adhesion test (seconds)	pH	Viscosity (cP)
1	5.73	1.06	4.72	1896
2	7.65	0.32	5.08	192
3	5.5	1.17	4.7	4692
4	5.65	1.08	4.76	3060
5	5.18	1.29	4.72	8724
6	4.8	1.89	4.53	11440
7	5.58	1.11	4.64	3180
8	6.43	0.54	5.01	984
9	6.78	0.35	5.11	420
10	5.93	0.92	4.61	2016
11	5.2	1.18	4.45	6864
12	6.18	0.73	5.59	1920
13	5.75	1.01	4.68	2304
14	6.7	0.45	4.87	624
15	6.23	0.71	4.82	2316
16	6	0.85	4.98	2016
17	6.2	1.01	4.82	1908
18	6.38	0.64	5.55	1296
19	6.45	0.5	5.23	948
20	3.48	2.54	4.86	11500
21	7.1	0.34	5.54	264
22	6.65	0.5	5.49	2004
23	6.05	0.76	5.16	804
24	5.5	1.11	5.42	3564
25	6.3	0.7	5.48	1632
26	5.85	0.95	4.96	2136
27	6.43	0.56	5.36	1032

The response model of adhesive power shows a 2FI model (p: 0.05, Model F-value of 8.37, Lack of fit (p: 5.25, Lack of Fit F-value of 8.53), R^2: 0.9195, Adjusted R^2 0.8096). These data indicate a fairly complex interaction with the adhesive power parameters. The dominant excipients in the adhesive power response

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include oleic acid and tween 80. Increasing the concentration of oleic acid will cause an increase in adhesive power, and increasing tween 80 will cause a decrease in the adhesive power of the emulgel. This is in line with the pharmaceutical concept that increasing the concentration of the lipid phase of the emulgel will increase the adhesive properties, while increasing the concentration of tween 80 decreases the cohesiveness of the emulgel.[18–20]

The response model of pH shows a linear model (p: 0.41, Model F-value of 4.87, Lack of fit (not significant), R^2: 0.5370, Adjusted R^2: 0.4268. These data indicate that oleic acid is the most dominant excipient and influences pH. Increasing the concentration of oleic acid will cause a decrease in the pH of the emulgel.[18–20]

The response model of viscosity shows a 2FI model (p: 0.31, Model F-value of 5.64, Lack of fit (p: 9.21, F-value of 5.60), R^2: 0.8849, Adjusted R^2: 0.7280. These data indicate a fairly complex interaction with viscosity parameters. The dominant excipient in the viscosity response is oleic acid. Increasing the concentration of oleic acid will cause an increase in viscosity. This is in line with the pharmaceutical concept when increasing viscosity will be linear to the increase in adhesiveness and inversely proportional to spreadability.[18–20]

DoE using the RSM – D-Optimal method provides a recommendation for an optimal CN-emulgel formula (desirability value: 0.504. The confirmation and analysis results of the optimum CN-emulgel are shown in Table 6. The desirability value of 0.504 is suspected to be caused by inconsistencies in the manufacturing process or the composition of the excipients used is not optimal. The manufacturing process, especially at critical points such as the temperature during the gelling agent development process, the speed of the homogenization process, and the temperature of the mixing process. These critical points in the manufacturing process affect the physical and chemical characteristics of the CN-emulgel preparation. The desirability value of 0.504 can also be affected by the unoptimized excipient composition. This CN-emulgel condition requires further optimization by narrowing the concentration range of each excipient. This further optimization will produce a better excipient composition with a higher desirability value.[2,18] The desirability value is an indicator in the optimization process that reflects the extent to which a solution meets the predetermined criteria to achieve the desired end product.[21]

Table 6. Confirmation and validation of optimum CN-emulgel

Composition	Formula CN-Emulgel (%)		
	Rep 1	**Rep 2**	**Rep 3**
HPMC	2	2	2
Glycerin	1	1	1
Tween 80	4.99	4.99	4.99
Propylene glycol	1.14	1.14	1.14
Oleic acid	14.84	14.84	14.84
Deionized water	ad 100	ad 100	ad 100
Characterization			
Spreadability test (cm)	3.94	3.53	4.28
Average ± SD		3.92 ± 0.38	
adhesion test (second)	2.15	2.47	1.89
Average ± SD		2.17 ± 0.29	
pH	4.73	4.61	4.82
Average ± SD		4.72 ± 0.11	
Viscosity (cP)	12270	12820	11060
Average ± SD		12050 ± 900.39	
Desirability value		0.504	

CN-Emulgel Release

The release of CN from the emulgel preparation is presented in Figure 3, compared to the CN solution preparation. The emulgel preparation provided a controlled and higher release than the solution preparation. The total release of CN from the emulgel for 180 minutes was 19.25% and from the CN solution was 14.59%. The release of CN-emulgel took place in a controlled or gradual manner from the 15th, 30th, 60th, 120th, and 180th minutes respectively at 2.14; 2.44; 3.57; 5.12; 5; 98% compared to cinchonine solution of 1.49; 2.05; 2.09; 4.07 and 4.87%. The increase in CN release in the emulgel was

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influenced by the emulgel delivery system used. The use of liquid lipid (oleic acid) in the lipid phase of the emulsion is thought to be the dominant factor and plays an important role in the permeability of CN across the dialysis bag membrane.[9] In this situation, the oleic acid excipient can act as a CN penetration enhancer. The use of a gelling agent (HPMC) is the preferred strategy for achieving controlled release. This controlled release is expected to minimize or reduce the risk of side effects that can be caused by spikes in systemic CN levels.[2]

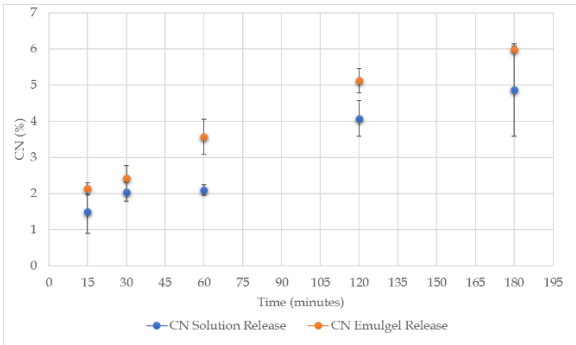

Figure 3. Release of CN-emulgel (n = 3)

Antibacterial activity test

Inhibition zone measurements were performed at 18- and 24-hour intervals to assess bacterial sensitivity to the tested antibacterials. This was done because antibiotic stability is a crucial factor influencing antibacterial pharmacological activity.[22] The results of the antibacterial activity test are presented in Figure 4. The antibacterial activity data for 18 and 24 hours respectively obtained from the CN-emulgel preparation against P. aeruginosa were 7.68±0.14 and 6.50±0.37 mm, S. aureus 10.47±0.37 and 10.36±0.69 mm. The inhibition zone was included in the moderate category of potency (5-10 mm) compared to the positive control (gentamicin, 18 hours: 18.15±0.90 and 24 hours 16.99±1.61 mm) and blank control (18 hours: 00.00±0.00 and 24 hours 00.00±0.00 mm) ($p<0.05$).[1] When the antibacterial activity of CN is compared to that of the blank group, this indicates that the blank or emulgel base has no antibacterial potential. According to the sizes of the inhibitory zone, the antimicrobial activity is graded as inactive (0–3 mm); mildly active (4–9 mm); moderately active (10–14 mm); and highly active (≥15 mm).[1,2] Inhibition zone data shows that CN has antibacterial potential against P. aeruginosa and S. aureus which meets the criteria for mild to moderate potency.[1] Thus, the antibacterial potency data for CN, meeting the mild to moderate potency criteria, represents an important milestone for this natural-based primary compound.[1,2] The antibacterial activity of CN, which is an alkaloid compound, is thought to occur through mechanisms including inhibition of cell wall formation (CN interacts with lipids and cell wall proteins)[23], modify membrane permeability or inhibition of enzyme activity.[24]

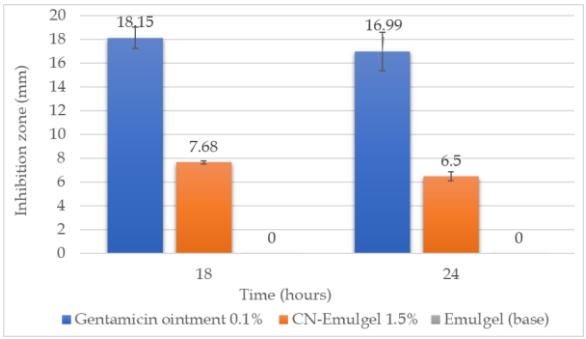

Figure 4. Antibacterial activity test (n = 3)

Stability Test

Stability testing was conducted using two methods: a cycling test to observe the effect of temperature (as shown in Figure 5) and an accelerated test to observe the effect of temperature-humidity on the physical stability of CN-emulgel (as shown in Figure 6). The results of the stability test for homogeneity and organoleptic parameters showed no significant changes in CN-emulgel. The CN-emulgel maintained its homogeneity, color, and odor according to its initial conditions. However, the stability test parameters in Figures 5 and 6 showed fluctuations in spreadability, adhesion, and pH. The fluctuations in these test parameters were not statistically significant, so neither temperature (cycling test) nor temperature-humidity

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(accelerated) caused instability in CN-emulgel. However, these data indicate that CN-emulgel requires a stability test with a longer storage duration. The pH fluctuation in the stability test is thought to be caused by high temperatures during storage which can affect the weakening of molecular bonds in HPMC[25], degradation of DMDM hydantoin which produces formaldehyde, oxidation of double bonds (C9-C10) of oleic acid.[26]

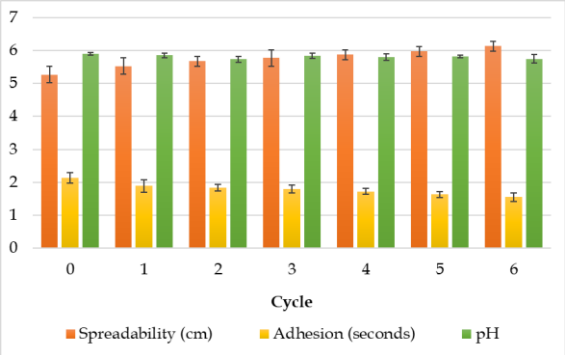

Figure 5. Cycling test stability test

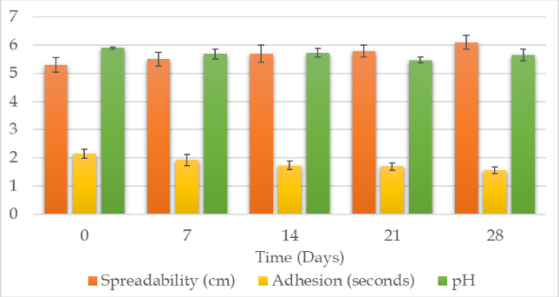

Figure 6. Accelerated stability test

Fluctuations in spreadability and adhesion are predominantly influenced by the gelling agent (HPMC). High temperatures (40°C) weaken the intermolecular bonds of HPMC, making it unable to maintain the consistency and viscosity of CN-emulgel. These changes in consistency and viscosity result in an increase in the spreadability of CN-emulgel. This increase in spreadability correlates with a decrease in the adhesive power of CN-emulgel.[2,25]

Skin irritation test

The data in Table 7 shows that CN and the excipients used in the CN-emulgel are non-irritant, as indicated by the primary irritation index parameter of 0.00.

Table 7. Skin irritation test

Rabbit	Group			
	Normal Control	Blank Control	Gentamicin	CN-Emulgel
Erythema	0	0	0	0
Edema	0	0	0	0
Erythema	0	0	0	0
Edema	0	0	0	0
Erythema	0	0	0	0
Edema	0	0	0	0
Erythema	0	0	0	0
Edema	0	0	0	0

All ingredients used do not show the potential to cause skin irritation, as seen from the erythema and edema parameters with a value of 0.00.[6,15] Based on the research data generated, further research is needed to obtain the shelf life, optimal storage conditions, in vivo antibacterial activity and reproducibility of CN-emulgel for production on a larger scale.

CONCLUSION

The proposed RSM formula showed acceptable physical properties and moderate antibacterial activity compared to the positive control (gentamicin) and blank control (emulgel base). The optimal CN-emulgel formula had a composition of CN 1.5; HPMC 2; glycerin 1, tween 80 4.99; propylene glycol 1; oleic acid 14.84, and deionized water up to 100%. CN-emulgel has a gradual release so it can minimize the occurrence of side effects. CN-emulgel does not cause irritation as indicated by the primary irritation index parameter of 0.00. CN-emulgel is stable during storage for ± 28 days. Further research is needed to determine the permeation pathway through the skin, and the safety profile with a larger number of groups.

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Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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