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by Shaum Shiyan

Submission date: 21-May-2023 08:17PM (UTC+0700)
Submission ID: 2098303612
File name: 3_RJPT_16_4_2023.pdf (460.29K)
Word count: 5616
Character count: 30305

ISSN 0974-3618 (Print)
0974-360X (Online)

www.rjptonline.org

RESEARCH ARTICLE

Factorial Design for the Optimization of Clindamycin HCl-Loaded Ethosome with various concentrations of Phospholipon 90g and Ethanol

Elsa Fitria Apriani[1]*, Shaum Shiyan[1], Iwi Hardestyariki[2], Viva Starlista[1], Mariska Febriani[1]

[1]Department of Pharmacy, Faculty of Mathematics and Natural Sciences, Sriwijaya University, South Sumatra, Indonesia.

[2]Department of Biology, Faculty of Mathematics and Natural Sciences, Sriwijaya University, South Sumatra, Indonesia.

*Corresponding Author E-mail: **elsafitria@mipa.unsri.ac.id**

ABSTRACT:

Clindamycin HCl is a drug for the treatment of acne. Clindamycin can act as an anti-acne by interfering with bacterial protein synthesis. The concentration of clindamycin used in the treatment of acne is 1%. Still, the bioavailability of the drug in the serum only reaches 0.7 – 12.4% of the total active substance in the preparation, so it is necessary to develop preparations that can increase the bioavailability of the drug Clindamycin HCl, one of which is in the form of ethosomes. This study aimed to optimize the clindamycin HCl loaded ethosomes formula with variations in the concentration of Phospholipon 90G and ethanol using the 22 factorial design method to obtain four formulas. The concentrations of phospholipon 90G used were 2% and 4%, while ethanol was 20% and 40%, respectively. Ethosomes were prepared using the thin layer hydration method and characterized by percent entrapment efficiency (%EE), particle size, and polydispersity index to determine the optimum formula. Based on the factorial design analysis results, the concentration of Phosholipon 90G, ethanol, and their two interactions significantly affected the value of entrapment efficiency, particle size, and polydispersity index with $p<0.05$. The optimum formula was obtained using 2% phospolipon 90G and 40% ethanol with an entrapment efficiency of 98.31±0.06, a particle size of 179.6±8.6nm, and a polydispersity index of 0.361±0.015. The optimum formula also showed good solubility in distilled water and acid solvents and good physical stability.

KEYWORDS: Clindamycin HCl, Phospholipon 90G, Ethanol, Factorial Design.

INTRODUCTION:

Clindamycin is an antibiotic that can work as either bacteriostatic or bactericidal, depending on the concentration of the drug, the site of infection, and the organism causing the disease[1]. Clindamycin is commonly used in treating infections caused by gram-positive bacteria, one of which is an anti-acne drug[2]. The gram-positive bacteria that cause acne is *Propionibacterium acnes* (*P. acnes*)[3]. Clindamycin can reduce the population of P. acnes in the skin surface and pilosebaceous follicles. Clindamycin HCl inhibits the 50S subunit ribosomal protein synthesis[4-6].

Received on 05.04.2022 Modified on 01.07.2022
Accepted on 09.09.2022
Research J. Pharm. and Tech 2023; 16(4):1561-1568.
DOI: 10.52711/0974-360X.2023.00255

The level of clindamycin used in the treatment of acne is 1%. Still, the bioavailability of the drug is only about 0.7 – 12.4%, so the clindamycin HCl is used repeatedly to produce an optimal effect[7]. Repeated use of antibiotics is very susceptible to causing resistance[8-10]. It is necessary to develop to increase the bioavailability of clindamycin. One of the developments that can be done is using nanotechnology-based delivery systems such as ethosomes.

Ethosomes are carriers capable of penetrating the skin. Ethosomes are composed of phospholipids and ethanol at high concentrations reaching 45%[11]. High ethanol concentrations cause the permeability of skin to increase so that the ability of the drug to penetrate the skin will increase[12]. Meanwhile, the presence of phospholipids in the ethosomes system helps drugs enter the skin by fusion between lipids in ethosomes and lipids in the skin[13]. In the manufacture of ethosomes, the

concentration of phospholipids and ethanol are essential components that need to be optimized to get good ethosomes characteristics. The characteristics of good ethosomes are high entrapment efficiency, particle size less than 1000 nm, and polydispersity index less than 0.5. Using phospholipids with high concentrations can cause a decrease in efficiency, but even at low concentrations will decrease the sorption efficiency[14,15]. The concentration of ethanol also influences the characteristics of the ethosomes. The higher the ethanol concentration, the smaller the particle size produced[16].

Based on the description above, the researchers will use nanotechnology in the clindamycin HCl delivery system in the form of ethosomes to increase its effectiveness as an anti-acne drug. This study was designed using a 2^2 factorial design which consists of two factors with two levels. The factors used are Phospholipon 90G and Ethanol. Specifically, the purpose of this study was to determine the optimum formula for clindamycin HCl-loaded ethosomes based on the results of data analysis on the effect of entrapment efficiency, particle size, and polydispersity index.

MATERIALS AND METHODS:

Materials:

The materials used in this study were clindamycin HCl obtained from PT. Dexa Medica (Indonesia), Phospolipon 90G (Lipoid®, USA), ethanol (Bratachem®, Indonesia), methanol (Bratachem®, Indonesia), dichloromethane (Bratachem®, Indonesia), propylene glycol (Smartlab®, Indonesia), buffer phosphate PH 7.4, aquadest (Smartlab®, Indonesia), NaOH (Emsure®, Indonesia), NaHCO3, HCl (Bratachem®, Indonesia), simulated gastric fluid (SGF), and simulated intestinal fluid (SIF).

Preparation of Clindamycin HCl Standard Curve:

The standard curve for clindamycin HCl was made according to Fadli et al.[17] with a few modifications. A total of 100 mg of clindamycin HCl was dissolved in phosphate buffer pH 7.4 to 100mL to obtain a concentration of 1000ppm and then made a series of concentrations of 80 to 120ppm. The concentration at 100ppm was used to determine the maximum wavelength at 200-400nm. The concentration series then measured the absorption using UV-Vis spectrophotometry at the specified maximum wavelength. Then made a linear regression equation Y = bx + a until the linearity value is close to 1.

Design Formula of Clindamycin HCl-Loaded Ethosome:

The clindamycin HCl-Loaded Ethosome formula was designed using the 2^2 Factorial Design, consisting of two factors with two levels. The concentration range of Phospholipon 90G refers to the study of Fathalla et al.[14], while ethanol refers to the research of Christiano et al.[16]. The design of the formula can be seen in Table 1.

Table 1. Formulation of Clindamycin HCl-Loaded Ethosome

Formula	Coded Level		Actual Level (%)		Clindamycin HCl	Phosphate Buffer pH 7.4
	Phospholipon 90G	Ethanol	Phospholipon 90G	Ethanol		
1	-1	-1	2	20	1	ad 100
2	+1	-1	4	20	1	ad 100
3	-1	+1	2	40	1	ad 100
4	+1	+1	4	40	1	ad 100

Preparation of Clindamycin HCl Loaded Ethosome:

The method used to manufacture clindamycin HCl-loaded ethosomes is a thin layer hydration method using glass beads[15]. Phospholipon 90G was dissolved in dichloromethane and methanol and then evaporated using a rotary vacuum evaporator. After forming a thin layer, let stand for 24 hours in the refrigerator. Then the thin layer formed was hydrated with a hydroethanolic solution of clindamycin HCl, which was made by dissolving 1% clindamycin HCl in ethanol. Furthermore, the solution was added with phosphate buffer pH 7.4 to a volume of 100ml. The resulting ethosomal suspension was reduced to particle size using ultrarurax at a speed of 8000rpm.

Characterization of Ethosome:

% Entrapment Efficiency:

The entrapment efficiency was achieved by an indirect method using a UV-Vis Spectrophotometer[18]. The clindamycin HCl ethosomal suspension was centrifuged at 15,000 rpm for 30 minutes at 4oC. The supernatant was taken, and the absorbance was measured at λmax of clindamycin. The clindamycin level was calculated based on the standard curve equation obtained, and then the percentage of entrapment efficiency was calculated based on the formula below:

%EE= (Qt-Qs)/Qt x 100%)

Information:

EE: entrapment efficiency

Qt: the theoretical amount of clindamycin in ethosomal suspension (μg/mL)

Qs: the amount of clindamycin detected in the supernatant (μg/mL)

Particle Size and Polydispersity Index:

Particle size and polydispersity index were measured using a Particle Size Analyzer (PSA). This measurement

uses an ethosomal suspension separated from free clindamycin HCl and then resuspended in ethosomal solvent. The suspension was diluted as much as 0.05 mL in 10 mL of phosphate buffer pH 7.4 then 1 mL was taken and put into a cuvette. After that, the ethosomal particle size and polydispersity index value were obtained[19].

Determination of the Optimum Formula for Clindamycin HCl loaded Ethosomes:

Determination of the optimum formula is carried out using the *Design-Expert*® program. The criteria for the optimum formula are the formulas with the highest percent entrapment efficiency, the smallest particle size, and the smallest polydispersity index value[20]. The system chose the optimum formula based on the desirability value close to 1.

Solubility Test for Optimum Formula:

Solubility tests were carried out using solvents with different pH, namely aquadest, 5% NaOH solution, 5% NaHCO3 solution, 5% HCl solution, simulated gastric fluid (SGF), and simulated intestinal fluid (SIF).

Stability Test for Optimum Formula:

Stability testing was carried out using the heating-cooling cycle method for six cycles at 4°C and 40°C. In the 0th and 6th cycles, organoleptic observations, pH, and percentage of entrapment efficiency were carried out[21,22].

Data analysis:

Data analysis on the characteristics of ethosomes was carried out using the *Design Expert*® Program. Data analysis was carried out by calculating the coefficient value of each factor and a combination of factors to obtain an equation of the relationship between factors and responses. Based on these equations, the effect of each factor and the interaction of the two factors on the measured response can be observed. Meanwhile, the stability test data was analysed using the SPSS® program using the paired t-test method.

RESULT:

Clindamycin HCl Calibration Curve:

Clindamycin HCl has a maximum wavelength of 206 nm. The calibration curve obtained in this study is y = 0.0057x - 0.0164 with a correlation coefficient (r) of 0.9946. This calibration curve equation was used to determine the clindamycin content and the percent entrapment efficiency.

Clindamycin HCl-Loaded Ethosome:

The ethosome obtained is a milky white suspension liquid with a characteristic ethanol odour, as shown in Figure 1. The clearest visible ethosomes are shown in EF3, followed by EF1, EF4, and then EF2. This clarity can be influenced by the level of solubility of clindamycin HCl to ethanol and can also be influenced by the different concentrations used in the formula. EF3 contains the highest ethanol and the smallest Phospholipon 90G.

Figure 1. The Result of Clindamycin HCl-Loaded Ethosome

Characterization of Clindamycin HCl-Loaded Ethosome:

The characterization of clindamycin HCl-loaded ethosome included entrapment efficiency, particle size, and polydispersity index. The results of the characterization can be seen in table 3.

Table 3: Characteristics Results of Clindamycin HCl Loaded Ethosomes

Parameter		EF1	EF2	EF3	EF4
R_1	Entrapment Efficiency (%)	97.12 ± 0.06	98.09 ± 0.02	98.31 ± 0.06	97.05 ± 0.02
R_2	Particle Size (nm)	869 ± 78.7	2637 ± 851	179.6 ± 8.6	190.6 ± 10.8
R_3	Polydispersity Index	0.863 ± 0.074	0.999 ±0.001	0.361 ± 0.015	0.431 ± 0.072

Based on the characterization results above, EF3 has the best characteristics where the percentage value of the entrapment efficiency is 98.31±0.06%, the smallest particle size is 179.6±8.6, and the smallest PDI is 0.361 ±0.015.

Model Analysis of Clindamycin HCl Loaded Ethosome Formula:

Model analysis was performed using the Design-Expert 12® program. Modelling for optimization can provide accurate results by evaluating parameters such as R2, adjusted R2, predicted R2, and adequate precision. A good model result will be obtained if it fulfils several parameters, including the R2 value is greater than 0.7, the difference value between adjusted R2 and predicted R2 is not more than 0.2, and the adequate precision value is more than 4[23]. The analysis of the clindamycin HCl-loaded ethosome formula model can be seen in Table 4.

Table 4: Model Analysis of Clindamycin HCl-Loaded Ethosome Formula

Respon		Parameter				
		Standard Deviation	R^2	Adjusted R^2	Predicted R^2	Adequate precision
R_1	Entrapment Efficiency	0.0364	0.9972	0.9961	0.9937	59.1616
R_2	Particle Size	411.54	0.8974	0.8589	0.7691	10.2642
R_3	Polydispersity Index	0.2866	0.9906	0.9871	0.9789	33.9214

The response of entrapment efficiency, particle size, and polydispersity index based on the evaluation results can be seen in Table 4, which has an R2 value of more than 0.7, the difference between adjusted R2 and predicted R2 is less than 0.2, and the adequate precision value is greater than 4. analysis, all responses indicate good results so that they can be used to predict the optimum formula.

Data Analysis of The Response:

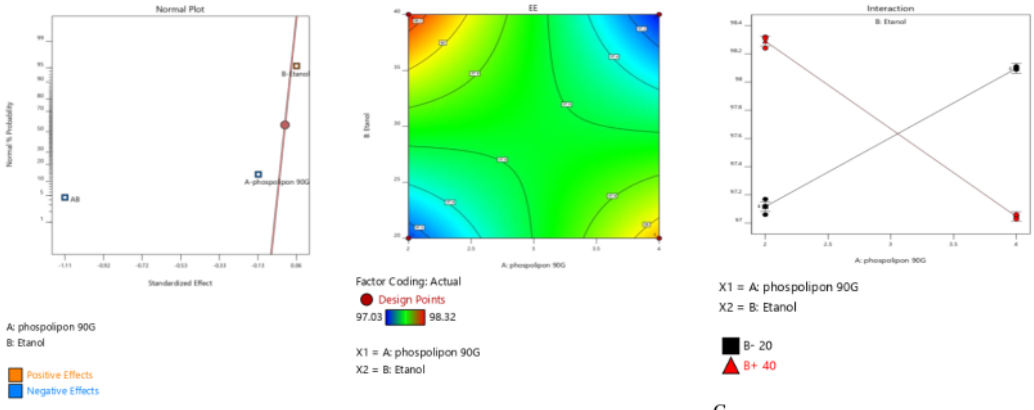
The response data were analysed to see the effect of each factor (concentration of Phospholipone 90G and Ethanol) and the interaction of the two factors on the response. The relationship between factors and responses can be seen in Table 5 and Figure 2.

Table 5: Data Analysis of The Response

Respon	Parameter	Intercept	A	B	AB
R_1	Coefficient	97.6375	-0.0658	0.0308	-0.5558
	p-value		0.0002*	0.0189*	< 0.0001*
	% Contributions		1.3753	0.3016	98.0426
R_2	Coefficient	964.983	440.16	-779.23	-313.62
	p-value		0.0060*	0.0002*	0.0067*
	% Contributions		17.6109	55.1927	16.9333
R_3	Coefficient	0.6535	0.0517	-0.2668	-0.0276
	p-value		0.0006*	<0.0001*	0.0188*
	% Contributions		3.5565	94.4964	1.0104

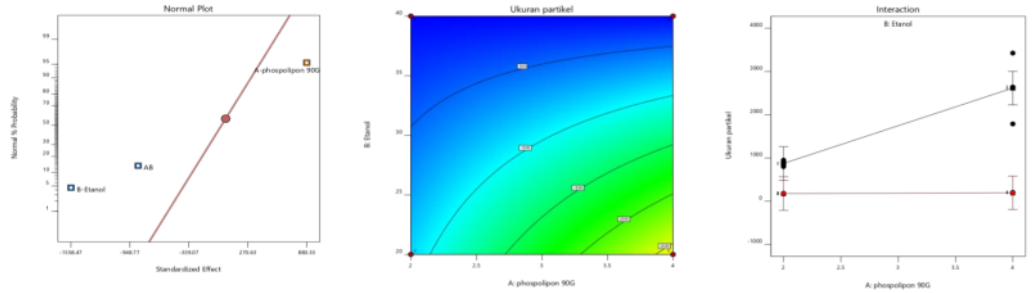
*p value <0.05 indicates that the factor has a significant effect on the responses

Entrapment Efficiency



A B C

Particle Size



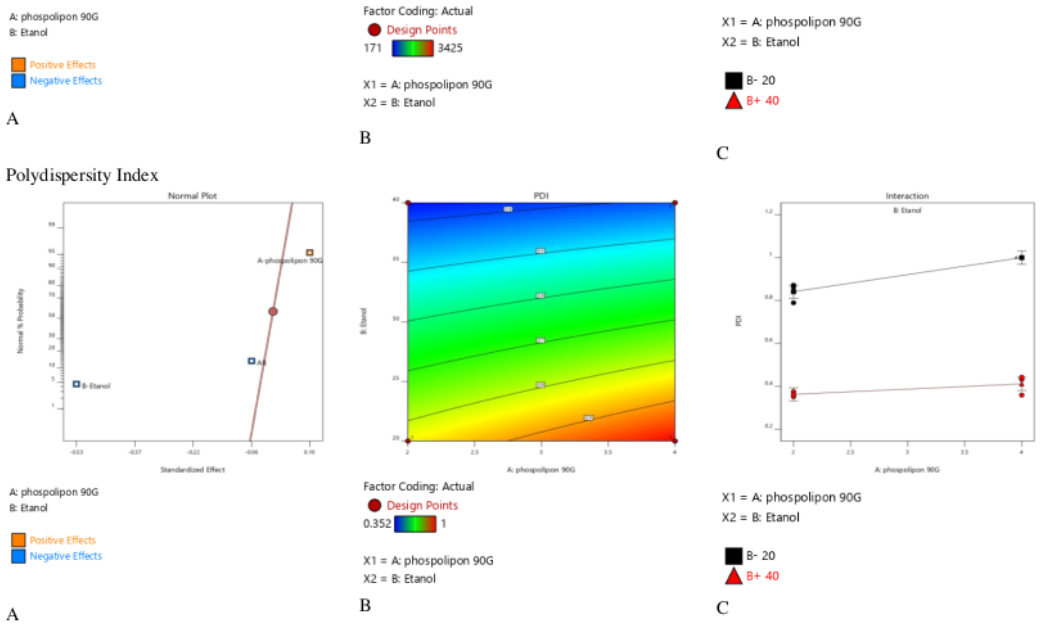


Figure 2: *(A) Normal Plot, (B) Contour Plot (C) Interaction Graph from Entrapment Efficiency, Particle Size and PolydispersityIndex*

The effect of Phospholipon 90G (A), Ethanol (B) and their interaction (AB) on the response can be described by the following equation.

$R_1 = 97.6375 - 0.0658A + 0.0308B - 0.5558\ AB$ (2)

$R_2 = 964{,}983 + 440{,}16A - 779{,}23B - 313{,}62\ AB$ (3)

$R_3 = 0{,}6535 + 0{,}0517A - 0{,}2668B - 0{,}0276AB$ (4)

Where : R_1 is Entrapment Efficiency Response; R_2 is Particle Size Response, R_3 is Polydispersity Index, A is Phospholipon 90G; B is Ethanol; and AB is Interaction between A and B

Formula Optimization

The *Design Expert* 12® program selected the optimum formula of clindamycin HCl loaded ethosome with a desirability value close to 1. The desirability value is a function value for optimization purposes that shows the program's ability to fulfil the wishes based on the criteria set on the final product. The optimum formula suggested by the system is a formula with a concentration of 2% phospholipon 90G and 40% ethanol with a desirability value of 0.986.

Solubility of Optimum Formula:

The test solution used is according to some of the fluids in the body. The purpose of using the test solution according to the fluid in the body is to see the suspension's efficiency and to determine the route of drug administration that is suitable for the body so that the clindamycin HCl-loaded ethosomes can reach the desired target effectively. The solubility of clindamycin HCl-loaded ethosomes is very soluble in water, HCl 5%, and Simulated Gastric Fluid (SGF), slightly soluble in NaOH 5% and Simulated Intestinal Fluid (SIF), and insoluble in NaHCO3 5%.

Stability of Optimum Formula:

Physical stability is very important to describe the resistance of the suspension containing clindamycin HCl-loaded ethosome during storage period and conditions. Based on the results of statistical analysis of the pH value and the percentage of entrapment efficiency, there was no significant difference between the 0th and 6th cycles ($p>0.05$). The pH value in cycles 0 and 6 was 7.25±0.02. The entrapment efficiency in cycles 0 and 6 was 97.0933±0.07 and 95.8911±0.15, respectively.

DISCUSSION:

The resulting ethosomes have the form of a milky white suspension liquid with a characteristic odor of ethanol. Ethosomes were prepared by the thin layer hydration method. The thin layer was made at the Phospholipone transition temperature of 90G, which is 54°C[5]. At the transition temperature, the lipid will change from the gel phase to a liquid phase with a higher permeability that can affect the entrapment of clindamycin HCl in the ethosome. After forming a thin layer, followed by the hydration process. The hydration process results incorporate phospholipids with other materials, thus forming a spherical shape resembling a ball due to the

attractive forces between equal parts of the phospholipids. Phospholipids consisting of a hydrophilic head and a hydrophobic tail will automatically join and lead their hydrophilic head to the surface in contact with the phosphate buffer, while the tail will be between the lamellar vesicles and form a hydrophobic layer[24]. In the hydration process, the phospholipids will encapsulate clindamycin HCl as shown in Figure 3.

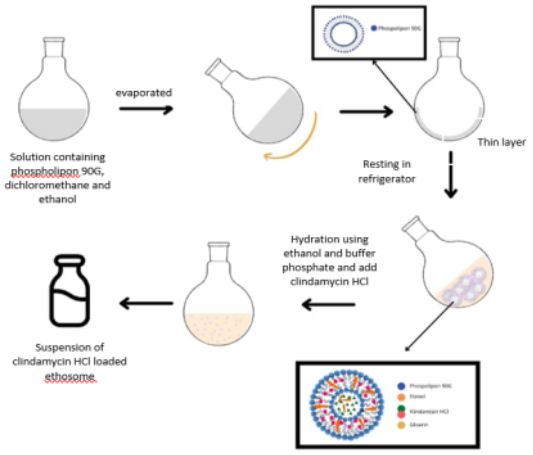


Figure 3. Formation Process of Clindamycin HCl Loaded Ethosome

The ethosome suspension is then characterized by measuring the percentage of entrapment efficiency, particle size, and polydispersity index. The characterization results can be seen in Table 3. EF3 has the best characterization results where the entrapment efficiency is the highest, the particle size is the smallest, the polydispersity index is the smallest. The higher the percentage of entrapment efficiency, the more clindamycin HCl was adsorbed in the ethosomal vesicles. The EF3 particle size of 179.6±8.6nm is said to be good and can increase the antibacterial activity of clindamycin HCl. Pei et al.[25] conducted an antibacterial activity test with a particle size of 135.8nm on several types of bacteria, which showed that the particles had strong antibacterial activity. In addition, Raza et al.[26] also showed that the size of 941nm still has good antibacterial activity. The polydispersity of EF3 of 0.361 ±0.015 indicates that the resulting particles are uniform. The value of the polydispersity index is directly proportional to the value of the particle size. The polydispersity index will affect clindamycin HCl's antibacterial activity by helping deliver uniform drug concentrations into the bacterial cytoplasmic membrane[27].

Before the optimization process is carried out, the model is analyzed first to ensure whether the model can be continued for the optimization process or not. Based on the model analysis results as shown in Table 4, the designed model provides a good analysis in terms of the R2 value, the difference between the predicted vs actual value, and adequate precision so the model can be continued for the optimization process.

Analysis of the response to entrapment efficiency, particle size, and polydispersity index was carried out by observing the p-value, coefficient, %contribution, and supporting graphs. The result can be seen in Table 5 and Figure 2. In the analysis of the response to entrapment efficiency, as shown in Table, Phospholipon 90G, ethanol, and their interactions have a significant effect, indicated by a p-value <0.05. When viewed from the coefficients of each factor and the interaction of the two factors, ethanol and the interaction have a negative effect. In contrast, Phospholipon 90G has a positive effect. The graph supports this in Figure 2A where ethanol and its interactions are in the negative region while phospholipon 90G is in the positive region. AB gave the most significant % contribution, namely 98.0426%, so the interaction of the two factors gave the most significant effect. Ethanol has a positive impact on entrapment efficiency so that when the ethanol concentration is increased, the entrapment efficiency will be even greater. Increasing ethanol concentration makes it easier for clindamycin HCl to enter through the gap in the lipid bilayer caused by the stretching of the lipid vesicle structure. However, when it reaches the optimum point, increasing the concentration of ethanol can cause the vesicles formed to be very permeable, so leakage can occur in the vesicles, resulting in lower entrapment efficiency[28]. Phospholipon 90G and the interaction of the two factors have a negative effect. The higher the concentration of phospholipon 90G or the interaction of the two factors, the entrapment efficiency will decrease. This is evidenced by Dhillon et al.[29], where the ratio of drugs to lipids with an active substance concentration of 1:2 gave better particle size and entrapment efficiency than the ratios of 1:3, 1:4, and 1:5. The use of high lipid concentrations will cause thickening of the vesicle layer, making it difficult for the active substance to be adsorbed due to decreased permeability[30]. When Phospholipon 90G and ethanol are used at the same high concentration, the entrapment efficiency will significantly reduce. This is supported by 2B and 2C images. In the contour plot image, the lowest entrapment efficiency is in the blue area with a concentration of 4% phospholipon 90G and 40% ethanol.

In the particle size response analysis, Phospholipon 90G, ethanol, and their interactions have a significant effect, indicated by a p-value <0.05. When viewed from the coefficients of each factor and the interaction of the two factors, ethanol and the interaction of the two factors

had a negative effect. In contrast, phospholipon 90G had a positive effect. The graph supports this in Figure 2A where ethanol and its interactions are in the negative region while phospholipon 90G is in the positive region. Ethanol gave the most significant contribution, namely 55,1927%. Ethanol has a negative effect because the higher the ethanol concentration, the surface of the vesicles will change and stretch so that the particle size will also decrease[31-33]. This is supported by Figures 2B and 2C where the same lipid concentration but different ethanol will give different particle size results. The resulting particle size will be smaller when ethanol is at a high concentration. Particle size will also affect the antibacterial action[34].

In the response analysis of the polydispersity index, the conclusion of the analysis is the same as in the particle size response analysis. Ethanol has a negative effect, and the most significant % contribution is 94,4964%. The polydispersity index value is directly proportional to the particle size value. The smaller the particle size, the smaller the polydispersity index value so that the resulting particles will be more uniform.

The optimum formula was then determined after the response analysis was carried out. The optimum formula was determined based on the criteria desired by the researcher, namely maximum adsorption efficiency, minimum particle size, and minimum polydispersity index. Based on these criteria, the system chooses Phospholipone 90G concentration of 2% and ethanol of 40% as the optimum formula from the desirability value of 0.986.

The optimum formula was then tested for solubility and stability tests. The solubility test results showed that the clindamycin HCl-loaded ethosomes suspension was very soluble in aquadest and a solvent with an acidic pH of 5% HCl and SGF. Meanwhile, the ethosomal suspension of Clindamycin HCl in alkaline solvents, namely 5% NaOH, 5% NaHCO3, and SIF has insoluble solubility. This is because the clindamycin HCl-loaded ethosomes suspension has a weak alkaline pH, so it is easier to form complexes and dissolve when mixed with a medium with an acidic pH compared to a medium with an alkaline pH. Based on the results obtained, it can be concluded that the clindamycin HCl-loaded ethosomes suspension has good bioavailability because it dissolves completely in distilled water[35]. Solubility at acidic pH indicates that clindamycin HCl-loaded ethosomes can be used by the oral route of administration because it can dissolve in the gastric acid medium so that the suspension bioavailability is good in gastric fluid[36,37]. The optimum formula also has good physical stability where there is no significant change in organoleptic, pH, and percentage of adsorption efficiency. In addition, no precipitate was formed, which indicated that the ethosomes were stable.

CONCLUSION:

In manufacturing clindamycin HCl-loaded ethosomes, the concentration of phospholipon 90G, ethanol, and the two interactions affected the characterization results based on factorial design analysis. On the response to entrapment efficiency the use of ethanol has a positive effect. In contrast, phospholipon 90G and the interaction of the two have a negative effect. Still, it differs from the response to particle size and polydispersity index where phospholipon 90G has a positive effect, while ethanol and their interactions have a negative effect. The optimum concentrations of phospolipon 90G and ethanol based on the results of factorial design analysis were 2% and 40%, respectively, with a desirability value of 0.986. EF3, which has the same concentration as the optimum concentration, gave the best characterization results, namely the entrapment efficiency of 98.31± 0.06%, particle size of 179.6±8.6nm, and PDI of 0.361 ±0.015. Solubility and stability of clindamycin HCl ethosomes from the optimum formula has good solubility and is physically stable.

CONFLICT OF INTEREST:

The authors have no conflicts of interest regarding this investigation.

FUNDING/ SUPPORT:

The research was funded by DIPA of the Public Service Agency of Universitas Sriwijaya 2022 SP DIPA-023.17.2.677515/2022 on December 13, 2021 in accordance with the Rector's Decree Number 0110/UN9.3.1/SK/2022 on April 28, 2022. PT. DKSH Indonesia (Malvern) and PT. Dexa Medica Indonesia also supported this study.

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FINAL GRADE

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GENERAL COMMENTS

Instructor

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RUBRIC: 6TH-8TH SCIENCE ARGUMENT (CER)

CLAIM

Take an arguable position on the scientific topic and develop the essay around that stance.

ADVANCED	The essay introduces a precise, qualitative and/or quantitative claim based on the scientific topic or text(s), regarding the relationship between dependent and independent variables. The essay develops the claim and counterclaim fairly, distinguishing the claim from alternate or opposing claims.
PROFICIENT	The essay introduces a clear, qualitative and/or quantitative claim based on the scientific topic or text(s), regarding the relationship between dependent and independent variables. The essay effectively acknowledges and distinguishes the claim from alternate or opposing claims.
DEVELOPING	The essay attempts to introduce a qualitative and/or quantitative claim, based on the scientific topic or text(s), but it may be somewhat unclear or not maintained throughout the essay. The essay may not clearly acknowledge or distinguish the claim from alternate or opposing claims.
EMERGING	The essay does not clearly make a claim based on the scientific topic or text(s), or the claim is overly simplistic or vague. The essay does not acknowledge or distinguish counterclaims.

EVIDENCE

Include relevant facts, definitions, and examples to back up the claim.

ADVANCED	The essay supplies sufficient relevant, accurate qualitative and/or quantitative data and evidence related to the scientific topic or text(s) to support its claim and counterclaim.
PROFICIENT	The essay supplies relevant, accurate qualitative and/or quantitative data and evidence related to the scientific topic or text(s) to support its claim and counterclaim.
DEVELOPING	The essay supplies some qualitative and/or quantitative data and evidence, but it may not be closely related to the scientific topic or text(s), or the support that is offered relies mostly on summary of the source(s), thereby not effectively supporting the essay's claim and counterclaim.
EMERGING	The essay supplies very little or no data and evidence to support its claim and counterclaim, or the evidence that is provided is not clear or relevant.

REASONING

Explain how or why each piece of evidence supports the claim.

ADVANCED	The essay effectively applies scientific ideas and principles in order to explain how or why the cited evidence supports the claim. The essay demonstrates consistently logical reasoning and understanding of the scientific topic and/or text(s). The essay's explanations anticipate the audience's knowledge level and concerns about this scientific topic.

PROFICIENT	The essay applies scientific reasoning in order to explain how or why the cited evidence supports the claim. The essay demonstrates logical reasoning and understanding of the scientific topic and/or text(s). The essay's explanations attempt to anticipate the audience's knowledge level and concerns about this scientific topic.
DEVELOPING	The essay includes some reasoning and understanding of the scientific topic and/or text(s), but it does not effectively apply scientific ideas or principles to explain how or why the evidence supports the claim.
EMERGING	The essay does not demonstrate clear or relevant reasoning to support the claim or to demonstrate an understanding of the scientific topic and/or text(s).

FOCUS

Focus your writing on the prompt and task.

ADVANCED	The essay maintains strong focus on the purpose and task, using the whole essay to support and develop the claim and counterclaims evenly while thoroughly addressing the demands of the prompt.
PROFICIENT	The essay addresses the demands of the prompt and is mostly focused on the purpose and task. The essay may not acknowledge the claim and counterclaims evenly throughout.
DEVELOPING	The essay may not fully address the demands of the prompt or stay focused on the purpose and task. The writing may stray significantly off topic at times, and introduce the writer's bias occasionally, making it difficult to follow the central claim at times.
EMERGING	The essay does not maintain focus on purpose or task.

ORGANIZATION

Organize your writing in a logical sequence.

ADVANCED	The essay incorporates an organizational structure throughout that establishes clear relationships among the claim(s), counterclaims, reasons, and evidence. Effective transitional words and phrases are included to clarify the relationships between and among ideas (i.e. claim and reasons, reasons and evidence, claim and counterclaim) in a way that strengthens the argument. The essay includes an introduction and conclusion that effectively follows from and supports the argument presented.
PROFICIENT	The essay incorporates an organizational structure with clear transitional words and phrases that show the relationship between and among ideas. The essay includes a progression of ideas from beginning to end, including an introduction and concluding statement or section that follows from and supports the argument presented.
DEVELOPING	The essay uses a basic organizational structure and minimal transitional words and phrases, though relationships between and among ideas are not consistently

	clear. The essay moves from beginning to end; however, an introduction and/or conclusion may not be clearly evident.
EMERGING	The essay does not have an organizational structure and may simply offer a series of ideas without any clear transitions or connections. An introduction and conclusion are not evident.

LANGUAGE

Pay close attention to your tone, style, word choice, and sentence structure when writing.

ADVANCED	The essay effectively establishes and maintains a formal style and objective tone and incorporates language that anticipates the reader's knowledge level and concerns. The essay consistently demonstrates a clear command of conventions, while also employing discipline-specific word choices and varied sentence structure.
PROFICIENT	The essay generally establishes and maintains a formal style with few possible exceptions and incorporates language that anticipates the reader's knowledge level and concerns. The essay demonstrates a general command of conventions, while also employing discipline-specific word choices and some variety in sentence structure.
DEVELOPING	The essay does not maintain a formal style consistently and incorporates language that may not show an awareness of the reader's knowledge or concerns. The essay may contain errors in conventions that interfere with meaning. Some attempts at discipline-specific word choices are made, and sentence structure may not vary often.
EMERGING	The essay employs language that is inappropriate for the audience and is not formal in style. The essay may contain pervasive errors in conventions that interfere with meaning, word choice is not discipline-specific, and sentence structures are simplistic and unvaried.