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Formulation Design and Evaluation of Hydrocortisone-Loaded Nanoemulsion and Nanoemulsion Gel for Topical Delivery

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Abstract. Hydrocortisone is a corticosteroid drug used for topical atopic dermatitis treatment. Hydrocortisone has solubility on low water and also has low bioavailability. This is made in the system of nanoemulsion and nanoemulsion gel. The manufacture of hydrocortisone nanoemulsion used palm oil as an oil phase with various concentration from 10%, 12%, and 14%. This study aims to know the physicochemical characteristic and in vitro release of nanoemulsion and nanoemulsion gel. Nanoemulsion of hydrocortisone made with the emulsification method. The result shows that nanoemulsion and nanoemulsion gel of hydrocortisone with various oil phase concentration was suitable with the specified specs. The result of the particle size test of nanoemulsion formulation 1 (374 nm), 2 (395 nm), 3 (468 nm). Meanwhile, the result of the nanoemulsion gel was formulation 1 (447,8 nm), (543,2 nm), 3 (516,8 nm). Flux of nanoemulsion in vitro release test formulation 1, 2, 3 were 5,54 ± 0,24; 3,45 ± 0,43; and 3,08 ± 0,07. Whereas the gel nanoemulsion result were 2,96 ± 0,06; 2,61 ± 0,21; and 2,39 ± 0,32. The conclusion of this study is the optimal concentration of palm oil was 10% and a gel basis on nano-emulsion not affected the release of hydrocortisone.

INTRODUCTION

One of the corticosteroid medications used in the treatment of atopic dermatitis is hydrocortisone. Hydrocortisone ($C_{21}H_{30}O_5$) is a corticosteroid group drug that has the power of antiallergic and anti-inflammatory. These drugs have low potency and are usually used to treat atopic dermatitis [1]. Based on the BCS (Biopharmaceutical Classification System) system it is explained that hydrocortisone belongs to the second class with high permeability and low dissolution rate [2]

Topical drug administration containing corticosteroid drug can be used to reduce atopic dermatitis disease which is a chronic skin disease. Topically administered medication is intended to allow the medicine to work on deeper layers of skin from the surface of the skin. Hydrocortisone is topically best used for skin inflammation because it has less strong anti-inflammatory potential and small side effects.

Currently, many drug delivery systems have been developed in the form of Transdermal Drug Delivery System (TTDS). This TTDS is known to increase drug bioavailability and maintain more extended plasma drug levels [3]. Nanoemulsion is one of the most contemporary TTDS examples in use today. The particle size of about 10-500 nm has a large surface area and low interface voltage so it can penetrate the skin quickly. One of the essential components of nanoemulsion formulation is the concentration of the oil phase, surfactant, and cosurfactant. The oil phase used in this research is palm oil because it can dissolve hydrocortisone well than other solvents [1].

The weakness of nanoemulsion is an intermediate product, where nanoemulsion has a liquid dosage form and needs special packaging to facilitate its use. One way to overcome this is the manufacture of gel nanoemulsion

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systems. It should be noted that the addition of a gel base in the gel nanoemulsion preparation may affect the rate of drug release. So, in this research will be done a comparison of nanoemulsion formulation and nanoemulsion gel hydrocortisone with the variation of concentration of palm oil phase 10%, 12%, and 14%. So it can be determined how much the level of palm oil and the influence of base gel on the chemical physics characteristics are appropriate and have optimal release results by using Franz Diffusion Cells method.

EXPERIMENTAL DETAILS

Material

Hydrocortisone (Indofarma), palm oil (Bratachem), tween 80 (Merck), propylene glycol (Merck), ethanol (Bratachem), nipagin (Pharmaceutical grade), nipasol (Pharmaceutical grade), glycerin (Merck), HPMC (Bratachem), solution Phosphate buffer pH 6 and 7.4 ± 0.05.

Formulation of Hydrocortisone NanoemulsionsAnd Gel Nanoemulsions

TABLE 1. Formulations of Hydrocortisone Nanoemulsion

Materials	Function	Concentration % (b/v)		
		Formulation 1 (F1)	Formulation 2 (F2)	Formulation 3 (F3)
Hydrocortisone	Active Materials	1	1	1
Palm oil	Oil Phase	10	12	14
Tween 80	Surfaktan	45	45	45
Ethanol	Co-surfactant	10	10	10
Propilen Glikol	Co-solvent	10	10	10
Buffer Phosphate pH 6	Solvent		ad 100	

Hydrocortisone was dissolved in the oil phase of Palm Oil and propylene glycol using a magnetic stirrer at a rate of 1000 rpm in the space of 30 min. Separately, a water phase comprising tween 80, ethanol, and phosphate buffer pH 6 was dissolved using a magnetic stirrer at a rate of 1000 rpm at 45 °C for 30 min. After the homogeneous water phase and the temperature decreases approximately 35 °C the oil phase is introduced into the aqueous phase by titration method and is homogenized using a magnetic stirrer at a rate of 1000 rpm for 60 min.

TABLE 2. Gel Base Formulation

Materials	Function	Concentration % (b/v)
HPMC 4000	*Gelling agent*	10
Gliserin	Humectant	5
Nipagin	Preservative	0,1
Nipasol	Preservative	0,01
Aquades free CO_2	Solvent	ad 100

HPMC 4000 is dispersed in CO^2-free aquadest. The comparison of HPMC and CO^2-free aquadest is 1: 8.5. After being left for an hour, HPMC is milled until the gel mass is formed. Then added nipagin, nipasol and glycerin. It is stirred until homogeneous. The prepared nanoemulsion was introduced into the gel base of 1% of 30 g. 0,03 g of a nanoemulsion of hydrocortisone added to 30 g of gel base. Then the nanoemulsion mixture and gel base were stirred using a magnetic stirrer at 500 rpm for 15 min.

Evaluation of Hydrocortisone Nanoemulsions and Gel Nanoemulsions Organoleptic Test

The organoleptic test is done by observing the clarity, sedimentation, discoloration and smell descriptive. Expected results are clear, odorless, no phase separation, and solution according to nanoemulsion specification [4].

pH Test

The pH test aims to determine the pH value of the preparations included in the acceptable pH range of 4.5-6.5 [4]. 1 g dosage measured pH using pH meter.

Physical Stability Test

The physical stability test is aimed to evaluate the stability of the room temperature (28 ºC ± 2 ºC), high temperature (40 ºC ± 2 ºC), and low temperature (4 ºC ± 2 ºC) for two weeks. Examination conducted in the last 2 weeks which includes organoleptic testing (discoloration, odor, phase separation and clarity) and pH of the preparation [10].

Type of Emulsion Test

Aim to know which type of preparation of nanoemulsion and gel nanoemulsion of hydrocortisone is included in the type of oil in water or water in oil. The blue methylene is dissolved in dosage over a glass object then observed under a microscope. If the preparation is an oil type in water (o/w) then the blue methylene will dissolve therein, if it is water in oil (w/o) then the blue methylene will cluster on the surface [7].

Particle Size Test

The particle size test aims to determine the intensity of the spatial distribution of the scattered light induced by the laser beam. Measurements were made using the Zetasizer (Malvern). The size of the particles that are eligible for nanoemulsion preparations is 10-500 nm.

Entrapment efficiency

Nanoemulsionhydrocortisone of 1 g was dissolved with phosphate buffer pH 7.4 ± 0.05 to 10 mL. Then centrifuged at 2500 rpm for 45 min. The supernatant of centrifugation was determined using a UV-Vis spectrophotometer. Interpretation of the results of the entrapment efficiency it between 85-115% for the active ingredient of the drug.

Release Test from Nanoemulsion and Gel Nanoemulsion of Hydrocortisone

The receptor compartment is filled with a phosphate buffer solution of pH 7.4 ± 0.05 about 13 mL which is maintained at about 37 ± 0.5 ºC and stirred using a magnetic stirrer at a minimum speed of 300 rpm. Giving cellophane is placed between the donor compartment and the receptor compartment. Samples were weighed as much as ±1 g to be applied to cellophane membranes. Then take samples at minute 0, 20, 40, 60, 80, 100, 120, 150, 180, 210, 240, 300, 360 as much as 3 mL of receptor compartment by using syringe. The samples were observed for their absorbance using a UV-VIS spectrophotometer at the maximum wavelength.

The result of a calculation has formed a curve of a relationship between the cumulative amount of disengaged hydrocortisone (μg/cm^2) to time root in steady state. From the resulting curve can be made a regression equation, the slope of the regression equation is the speed of quercetin release (base) from the base [5].

RESULT AND DISCUSSION

Atopic dermatitis (DA) is caused by several factors that cause genetic and environmental factors. DA can be overcome with the help of topical drugs. Corticosteroid drugs can be used as topical drugs for DA, one of which uses hydrocortisone. Hydrocortisone functions as an anti-allergic and anti-inflammatory and has a low potential which causes little side effects. Topical administration of hydrocortisone can be done with conventional preparations or TTDS (Transdermal Drug Delivery System). Nanoemulsions have a minimum particle size that has a large surface, is more stable and has high bioavailability so that it can penetrate the skin too big. Nanoemulsion is formulation using palm oil as the oil phase, Tween 80 and ethanol as surfactant and cosurfactant [3]. Propylene glycol as cosolvent and phosphate buffer pH 6 as a solvent. The formulation of hydrocortisone nanoemulsion consists of three variations of the oil phase which are 10%, 12%, and 14%. In addition to the nanoemulsion formulation, in this study, a nanoemulsion gel formulation with the same composition and the same oil phase variation with the gelling agent

consisted of HPMC 4000, glycerin as a humectant, nipagin, and nipasol as the preservative, and CO^2 free aquades as a solvent. From each of the individual preparations, the characteristic test was carried out which included the organoleptic test, characteristic pH test, physical integrity test, emulsion type test, particle size test, and adsorption efficiency test. In addition to the characteristic test, the drug release test was carried out by the Franz diffusion cell method.

Nanoemulsion is a drug delivery system consisting of an oil and water emulsion system with an average droplet diameter ranging from 50 to 1000 nm, the average nanoemulsion droplet size is between 100 and 500 nm and is present as a form of oil in water (o/w) or water in oil (w/o), where the core of each particle is oil or water [4].

Organoleptic Test

Nanoemulsions and hydrocortisone nanoemulsion formulations have the same relative characteristic that is typical of tween 80, no phase separation, cloudy, and white color caused by active ingredients. The organoleptic nanoemulsion test and hydrocortisone gel nanoemulsion were carried out at week 0. The preparation of nanoemulsion blanks is bright yellow, smells typical of tween 80 because it contains tween 80 which is quite high, liquid, and no phase separation. Organoleptic testing on the gel base of HPMC 4000 has a transparent, odorless and homogeneous color. This indicates that the HPMC gel base to be used is following the standard so that it can be mixed with nanoemulsion to produce nanoemulsion gel. Organoleptic testing in the preparation of transparent, odorless and homogeneous colored nanoemulsion gel. While organoleptic testing on hydrocortisone gel nanoemulsion is white, odorless, and there is no phase separation. The results of organoleptic testing on nanoemulsion and nanoemulsion gel with variations in oil concentration between formulations 1, 2 and 3 gave the same organoleptic results.

pH Test

The pH test results showed that all nanoemulsion and nano-hydrocortisone hydraulic meet the pH criteria that can be tolerated to the skin. Differences in pH values in the three formulations of both nanoemulsion and gel nanoemulsion were not significantly different. The higher the concentration of palm oil, the more the pH value will decrease as the more the number of fatty acids in the preparation so that the amount of H^+ dissociates is more significant [12].

TABLE 3. The results of the pH test of hydrocortisone nanoemulsion

The pH of hydrocortisone nanoemulsion			
	F1	**F2**	**F3**
R1	5,80	5,50	5,50
R2	5,60	5,80	5,30
R3	5,60	5,70	5,40
Average ± SD	5,67 ± 0,11	5,67 ±0,15	5,40 ± 0,10

TABLE 4. The results of the pH test of hydrocortisone gel nanoemulsion

The pH of hydrocortisone gel nanoemulsion			
	F1	**F2**	**F3**
R1	5,60	5,50	5,70
R2	5,30	5,70	5,40
R3	5,80	5,30	5,20
Average ± SD	5,57 ± 0,25	5,50 ± 0,2	5,43 ± 0,25

Physical Stability Test

The results of the stability test show that nanoemulsion is stable at room temperature and high temperature. While nanoemulsion gel was only durable at room temperature. Nanoemulsion and gel nanoemulsion is unstable at low temperatures due to the oil phase of palm oil containing high saturated fatty acids so that the freezing point and the melting point are also higher [8]. While the gel nanoemulsion at high temperature is unstable arena occurs phase separation between nanoemulsion and gel base. The pH test on nanoemulsion and gel nanoemulsion at room temperature, high temperature, and low temperature decreased but the pH value change was still within the pH range of the skin, and there was no significant difference between the three nanoemulsion preparations and gel nanoemulsion.

Emulsion Type Test

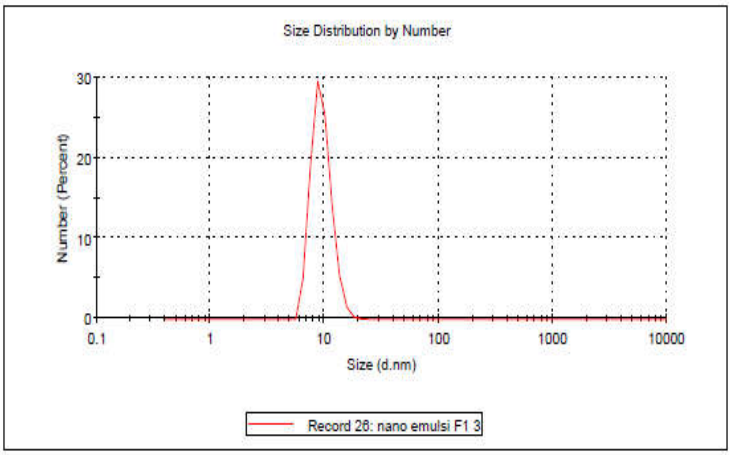
The nanoemulsion type test was carried out by dripping methylene blue in the preparation on the glass object then observed using an electron microscope with 400x magnification. The use of methylene blue is because methylene blue is a water-soluble substance. If methylene blue is dispersed evenly throughout the preparation, this test shows that the development is a type of oil nanoemulsion in water (o/w). But if methylene blue is not evenly dispersed or clustered, the test shows that the preparation has a water-in-oil nanoemulsion type (o/w) [5]. The results of observations on nanoemulsion type showed that methylene blue was dispersed evenly throughout the preparation. This indicates that the hydrocortisone nanoemulsion preparation in this study has an oil type in water (o/w).

FIGURE 1. Nanoemulsion type test results using electron microscopy

The type of nanoemulsion depends on the concentration and chemical properties of surfactants, oils, and materials dissolved in it. Surfactants that have polar groups tend to be stronger to form oil types in water[7]. In the formulation of nanoemulsion and nanoemulsion gel used tween 80 surfactant which is hydrophilic and the concentration of oil in the dosage formulation is lower than that of the water so that the type of emulsion produced is oil in water. Besides, the use of ethanol which is polar as cosurfactant can increase the kind of preparation emulsion in the form of oil in water (o/w).

Particle Size Test

The particle size assay results in the three nanoemulsion formulations meet the specified particle size, i.e. 10-500 nm. Particle size assay result for nanoemulsion of hydrocortisone are formulation1: 373.43 nm; formulation 2: 399.53 nm; and formulation 3: 468.97 nm. Particle size assay result for gel nanoemulsion of hydrocortisone are formulation 1: 447.87 nm; formulation 2: 543.27 nm; and formulation 3: 516.83 nm. While in gel nanoemulsion, only formulation 1 is a formulation containing 10% palm oil which has a particle size that meets the specified standard.



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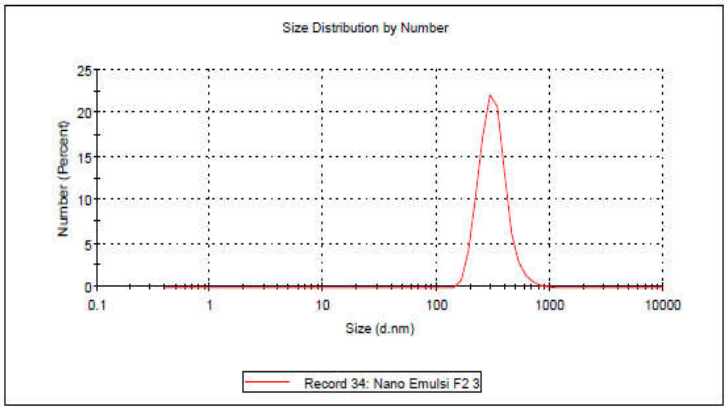

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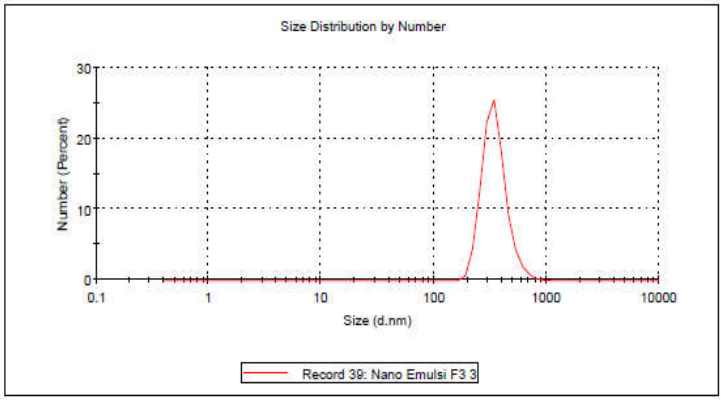

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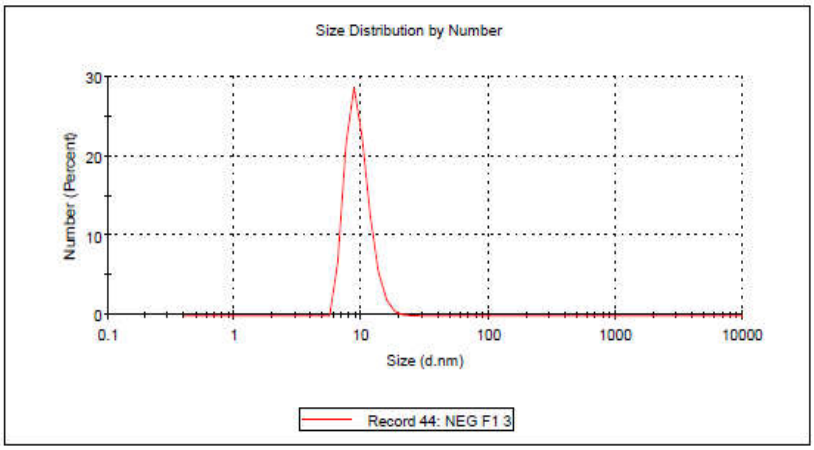
FIGURE 2. Graphs of measurements of nanoemulsion particles (a) Nanoemulsion F1 R3; (b) Nanoemulsion F2 R3; (c) Nanoemulsion F3 R3

The particle size of hydrocortisone nanoemulsion in formulation 1, formulation 2, and formulation 3 has particle size values that correspond to the nanoemulsion size range. Smaller size particles (nanosize) have advantages compared to large (macro) particles as a drug delivery system. Generally, nanoparticles have full absorption compared to microparticles, because the smaller the particle size, the larger the surface area of the particle, so that the intake is even more full [6].



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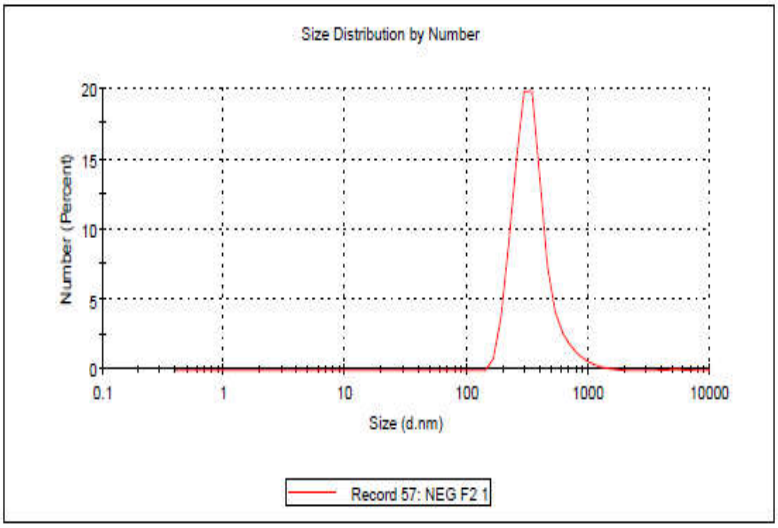

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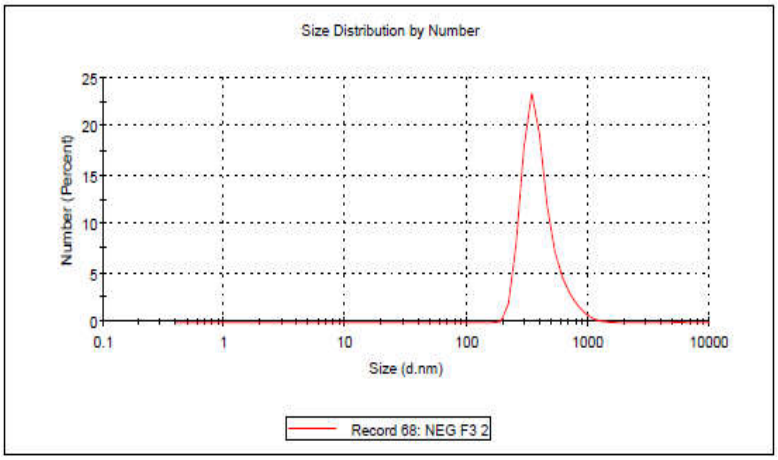

(c)

FIGURE 3. Graphs of measurements of gel nanoemulsion particles (a) Nanoemulsion gel F1 R3; (b) Nanoemulsion gel F2 R3; (c) Nanoemulsion gel F3 R3

Based on the graph, formulation 1 has the smallest particle size than formulation 2 and formulation 3. This is because in formulation 2 and formulation 3 the oil phase concentration increases, but the emulsifier concentration remains so that the oil phase dispersed into the water phase also decreases. As a result, the particle size is getting

bigger too. The smaller the particle size of the drug, the higher the surface area of the drug particles. So that the absorption of drugs in the body is higher and biological targets are broader compared to medicines that have a macroparticle size [6] The particle size of the nanoemulsion gel was also more significant than that of the nanoemulsion. This can be caused by several factors such as the length of stirring and the speed of mixing [7]. Based on the results of measurement tests on nanoemulsion gel preparations, only formulation 1 has the appropriate particle size which is still in the range of 10-500 nm. While formulations 2 and 3 are not included in the nanoemulsion size range because they have a particle size of more than 500 nm.

Entrapment Efficiency

A transdermal preparation is said to meet the requirements of the level if the level of the active ingredient in the development is 85% -115% while for the active ingredient derived from natural ingredients the content requirements are 50% -100%. Based on the calculation of entrapment efficiency on nanoemulsion preparations F1, F2, and F3 respectively 97,33%; 95.87%; and 95.13%. While on gel nanoemulsion F1, F2, and F3 that is 95,93%; 93.87%; and 92.53%. Based on these results indicate that all nanoemulsion and nanoemulsion gel formulations meet the specified percentage requirements. The higher the concentration of the oil phase in the preparation the lower the trap presentation. This may be due to the formulation with the lowest oil concentration having the smallest particle size so that the nanoemulsion particles are readily dispersed into a phosphate buffer pH 7.4 solution. Traffic efficiency is also increasing [1].

Release Test

The release test aims to determine the amount of hydrocortisone released through membrane cellophane per unit area and each time unit. Hydrocortisone released from the base will go to the receptor compartment through the cellophane membrane. Hydrocortisone released from the test results was then measured using a UV-Vis spectrophotometer through measurements of hydrocortisone absorbance at a wavelength of 427.60 nm.

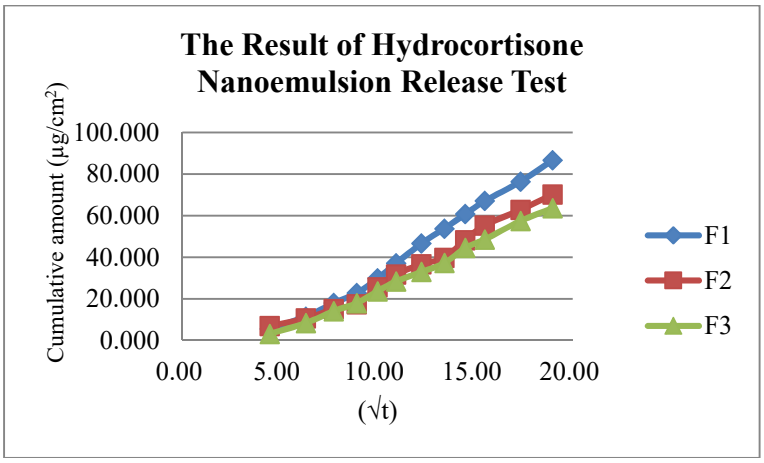

FIGURE 4. The result of release test hydrocortisone nanoemulsion

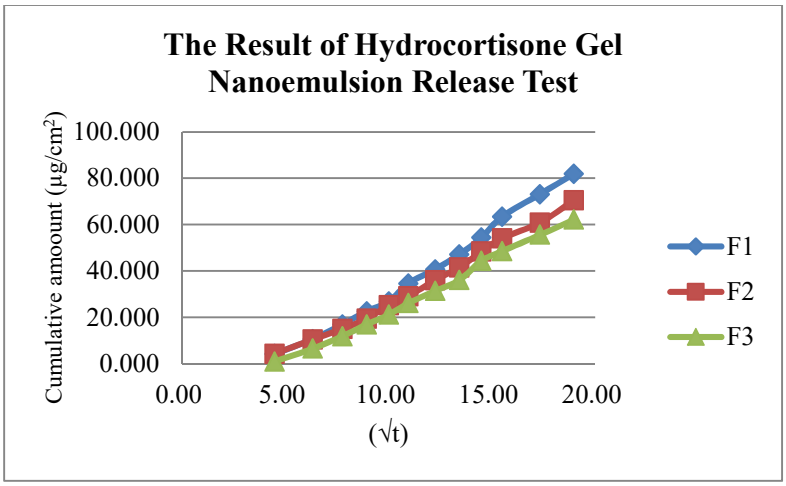

FIGURE 5. The result of the release test hydrocortisone gel nanoemulsion

Thus it can be determined the hydrocortisone release flux value in the nanoemulsion and gel nanoemulsion preparations. The flux value is the slope of the regression result between the mass of transport per unit area against time at steady state condition.

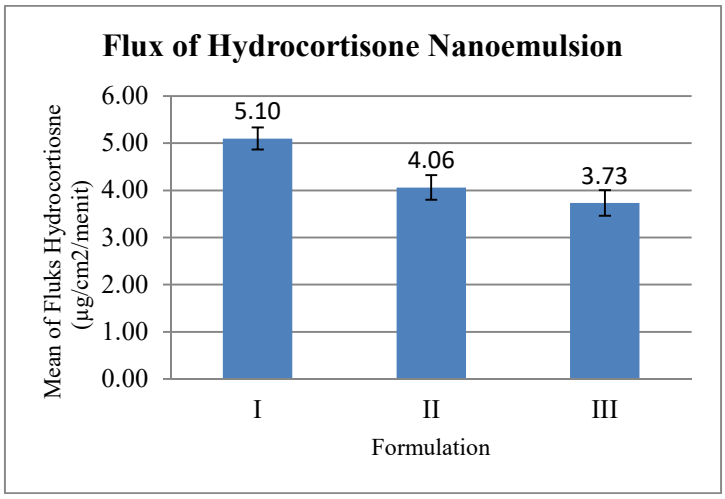

FIGURE 6. The result of flux release hydrocortisone nanoemulsion

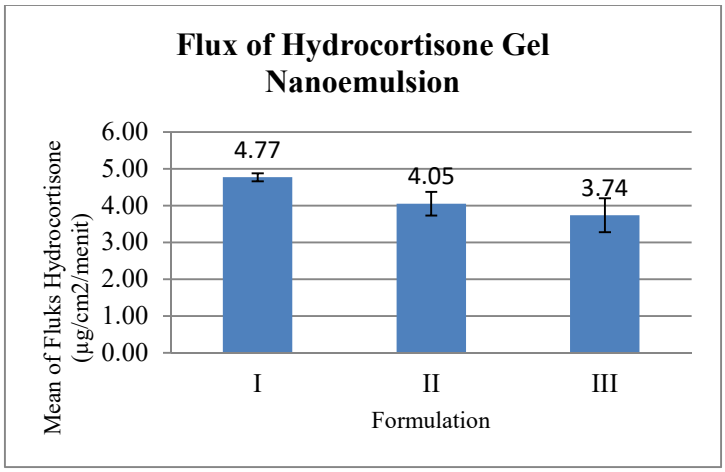

FIGURE 7. The result of flux release hydrocortisone gel nanoemulsion

The result of data analysis using One Way ANOVA showed that between the formulation of both nanoemulsion and nanoemulsion gel have a significant difference that is 0.002 for nanoemulsion and 0.022 for nanoemulsion gel. Both have a p-value <0.05, as for knowing whether there is a difference between the nanoemulsion preparation and gel nanoemulsion, a statistical test using Paired t-test was performed. Obtained a value of 0.423 (p-value> 0.05) indicating that there was no significant difference between nanoemulsion and gel nanoemulsion. So it can be said that the gel base does not affect the release of hydrocortisone nanoemulsion.

Formulation 1 in nanoemulsion and gel nanoemulsion has the highest release rate among other formulations. This is because formulation 1 has the smallest particle size compared to different formulations so that hydrocortisone can release well. The lower the particle size, the easier it is to provide high absorption efficiency at various delivery routes [8].

Based on the results of the release of nanoemulsion and hydrocortisone gel nanoemulsion showed that nanoemulsion preparations had a higher release value than nanoemulsion gel. This can be influenced by three main factors that determine the efficacy of the transdermal drug, namely the movement of the drug in its carrier, the release of the drug in its carrier, and the release of drugs in the skin [9]. The viscosity of the preparation influences the change of the drug in the carrier. The viscosity of nanoemulsion was lower than the thickness of nanoemulsion gel. This causes more significant movement of hydrocortisone in nanoemulsion compared to nanoemulsion gel so that hydrocortisone is quickly released. This shows that viscosity affects the release of hydrocortisone from the preparation.

Particle size can affect the amount of drug that can penetrate the skin. The smaller the particle size, the higher the number of drugs in the area of the stratum corneum, so that the number of medications released is even higher [10]. Based on the test results obtained, formulation 1 in nanoemulsion and gel nanoemulsion preparations which have smaller particle sizes have higher flux values. This is in line with the stated previous research that nanoemulsion has a minimal quantity of globule that can penetrate more quickly into the stratum corneum [11].

SUMMARY

There is a characteristic difference between nanoemulsion and nanoemulsion gel hydrocortisone with a variation of concentration of palm oil phase. Nanoemulsion and hydrocortisone gel nanoemulsions have stable physical and chemical characteristics of the organoleptic, pH characteristics, emulsion type, particle size, and defined trapping efficiency. But for physical stability test, hydrocortisone gel nanoemulsion is unstable in high temperature. The nanoemulsion in the gel base does not affect the release of hydrocortisone in the nanoemulsion system because the statistical test results show a value of 0.423 (p-value > 0.05) so that there is no significant difference of hydrocortisone flux rate in nanoemulsions and gel nanoemulsions.

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