Medical Sains : Jurnal Ilmiah Kefarmasian
Vol. 9 No. 4, October - December 2024
https:/ojs.ummada.ac.id/index.php/iojs

FORMULATION AND IN VITRO PENETRATION TESTING OF NUTMEG SEED OIL (*Myristica fragrans* Houtt.) NANOEMULGEL

Nashfiatun Nur Lailah[1], Fauziah Ningrum Syaputri[1], Rizky Dwi Larasati[2*], Hana Anisa Fatimi[3], Devi Yanthre Sari Manurung[2]

[1]*Program Studi Farmasi, Fakultas Sains dan Teknologi, Universitas Muhammadiyah Bandung, Bandung*
[2]*Program Studi Pendidikan Apoteker, Fakultas Matematika dan Ilmu Pengetahuan Alam, Universitas Sebelas Maret, Surakarta*
[3]*Program Studi Farmasi, Fakultas Matematika dan Ilmu Pengetahuan Alam, Universitas Sebelas Maret, Surakarta*
**Email Corresponding: rizkydl@staff.uns.ac.id*

Submitted: September 30, 2024 Revised: Desember 5, 2024 Accepted: Desember 6, 2024

ABSTRACT

Nutmeg seed oil has a low absorption time and is unstable during storage; therefore, so to facilitate this it is made in a nanoemulgel form because it can increase contact time and increase drug penetration into the skin. This study aimed to determine the effect of variations in nutmeg seed oil concentration on the physical characteristics and penetration rate of nanoemulgel preparations. Nanoemulgel was made by mixing the nanoemulsion phase into a gel base with oil concentrations of 4.5%, 5%, and 5.5%. The nanoemulsion physical characteristics results are 90.7–91.1% for the turbidimetry test, 453–516 nm for particle size, 42.4–48.6 mV for the zeta potential test, 6.46–6.66 for the pH test, and O/W for nanoemulsion type. Physical characteristics included organoleptic tests, homogeneity, pH, adhesive power, spreadability, viscosity, and rheology. The penetration rate was determined using the Franz diffusion-cell method. The results showed that the nanoemulgel preparation was white in color and had a characteristic smell of nutmeg oil, a semisolid, homogeneous texture, skin pH of 4.5–6.5, spreadability met the range of 5–7 cm, adhesion of >4 seconds, viscosity of 4000–50000 cPs, and pseudoplastic flow properties. The best formulas are found in Formulas 1 and 2 because the stability test meets the requirements for good physical characteristics. Variations in the concentration of nutmeg oil did not show any influence on the penetration rate, with the flux values obtained for each formula being F1 0.0117 mg/cm^{2}hour^{-1}, F2 0.0126 mg/cm^{2}hour^{-1}1, and F3 0.0246 mg/cm^{2}hour^{-1}.

Keywords: *Nutmeg seed oil, nanoemulgel, penetration*

INTRODUCTION

The nutmeg plant is a type of Indonesian spice that is a leading commodity in the agricultural sector and has abundant benefits, with promising selling value (Fitra *et al*., 2021). The parts of the nutmeg plant that are widely used are the fruit, seeds, and flowers because they can produce essential oil. Essential oils, such as nutmeg essential oil, have a distinctive aroma with diverse benefits. Nutmeg essential oil contains terpenoids, alkaloids, and flavonoids, which act as anti-inflammatory agents (Fitra *et al*. 2021). Inflammation is a protective response from a location that arises due to tissue damage caused by physical trauma, chemicals, or microbiological substances (Agustina *et al*., 2015). The essential oil content of nutmeg seeds, which can provide an anti-inflammatory effect, is 5%, which was obtained from the results of tests carried out on human fibroblast cell cultures (Matulyte *et al*., 2020). Therefore, in this study, the nutmeg seed oil concentrations used were 4.5, 5, and 5.5%.

Nutmeg seed oil is volatile, has a relatively low absorption time from the skin, and is unstable during storage (Shabrina *et al.* 2021). This limits their penetration into the skin. Only small molecules with sizes <500 Da and lipophilic properties with log P values between 1 and 3 can penetrate the stratum corneum (Kim *et al.*, 2012). Therefore, a delivery system such as a nanoemulsion is needed to enhance the penetration of nutmeg seed oil into the skin, thereby enhancing its effectiveness. Tween 80 can be used as a surfactant to reduce the size of the globules of lipophilic substances. In a study by Shabrina *et al.* (2021), sea buckthorn oil nanoemulsions were obtained with Tween 80 concentrations of 35–45%, producing a stable nanoemulsion for 90 days of storage. Tween 80 is a nonionic surfactant that is safer (less toxic) than ionic surfactants. They are minimally affected by pH and biocompatibility and have lower critical micelle concentration (CMC) values (Keservani and Sharma, 2019). Nanoemulsions have a good level of stability and transparency, but they are uncomfortable to use on the skin and have a low viscosity. Therefore, gel is added to increase its viscosity and comfort (Damayanti *et al.*, 2019). The advantages of nanoemulgel preparations are that they do not leave stains and are easy to clean, acceptable, transparent, and long-lasting (Ikhtiyarini and Sari, 2022). The disadvantages of nanoemulgel preparations are that they can form bubbles when formulated and have poor permeability (Dhawas *et al.*, 2020). Nanoemulgels are semisolid, have a soft texture, and are easily spread on the skin (Eid *et al*., 2014).

To facilitate the permeability of nutmeg oil, it is made in the form of nanoemulgel, so the smaller the particle size, the greater the surface area, which can increase penetration into the skin. In addition, small particle sizes can prevent separation and flocculation (Damayanti *et al*., 2019). Based on the description above, this study aims to develop a physically stable nutmeg oil (*Myristica fragrans* Houtt.) nanoemulgel formula and determine the penetration speed of nanoemulgel made at different concentrations of nutmeg oil (*Myristica fragrans* Houtt.).

RESEARCH METHODS

Equipment and Materials.

The equipment used includes Stirring Rods (Pyrex), Glass Funnels (Pyrex), Desiccators (Duran), Mortar and Pestle (D&N), Analytical Balance (Ohaus Navigator), Water Bath (Maspion), Wooden Clamp (D&N), pH Meter (Trans Instrument), Pycnometer (Pyrex), Refractometer (Macherey-Nagel), UV-Vis Spectrophotometer (Shimazu), and RION viscometer (Ika Ratavisc).

The materials used include Distilled water (UD. Kimia Market, Indonesia), BHT (Merck, Indonesia), ethanol 90% (PT. Brataco, Indonesia), and carbopol 940 (PT. Brataco, Indonesia), Whatman filter paper No. 1 (Merck, Indonesia), methylene blue (Merck, Indonesia), methylparaben (UD. Kimia Market, Indonesia), and nutmeg oil (CV. Ratu Aroma, Indonesia), propylparaben (UD. Kimia Market, Indonesia), and polyethylene glycol (UD. Kimia Market, Indonesia), and TEA (CV. Subur Kimia Jaya, Indonesia), tween 80 (CV. Subur Kimia Jaya, Indonesia), and Glycerin (CV. Subur Kimia Jaya, Indonesia).

Research Procedure

1. Sample Preparation

   The nutmeg oil used in this study was obtained from CV. Ratu Aroma, Cisoka, Tangerang Regency, Banten.

2. Test the Characteristics of Nutmeg Oil.

   Nutmeg oil was obtained from CV. Ratu Aroma was characterized according to SNI 06-2385-2006 including determining color and odor, specific gravity, refractive index, solubility in ethanol, optical rotation, and evaporation residue.

3. Preparation of Nanoemulsion Preparations

   The nanoemulsions were prepared using a low-energy method. Nutmeg oil is an active substance that can act as an anti-inflammatory agent at various concentrations. F1 contained 4.5% nutmeg oil, F2 contained 5% nutmeg oil, and F3 contained 5.5% nutmeg oil, as shown in Table I, as follows:

Table I. Nutmeg Seed Oil Nanoemulsion Formulation

No	Material	Formulation (Gram)		
		F1	F2	F3
1	Nutmeg Seed Oil	4.5	5	5.5
2	Tween 80	45	45	45
3	Propylparaben	0.02	0.02	0.02
4	Polyethylene glycol	30	30	30
5	BHT	0.1	0.1	0.1
6	Aquades	Ad 100	Ad 100	Ad 100

The nanoemulsion was prepared by heating distilled water to 30 °C. After that, propylparaben and BHT were dissolved in Span 80 while heating and stirring until dissolved, and propylene glycol and methylparaben were dissolved in Tween 80. The mixture was stirred at 1500 rpm at 30ºC until it became homogeneous. Once homogeneous, heated distilled water was added while stirring, until a clear solution was obtained. Nutmeg oil was then added dropwise while stirring using a magnetic stirrer at 1500 rpm and 30ºC (Shabrina *et al*., 2021).

4. Evaluation Test of Nutmeg Oil Nanoemulsion
   a. Turbidimetry

      Turbidimetry testing was performed to observe the opacity of the nanoemulsion preparation using a UV-Vis spectrophotometer at a maximum wavelength of 650 nm with a distilled water blank. The ideal value for nanoemulsions is in the range–90-100%. This range includes optimal visual nanoemulsions because they show clear and transparent visuals (Indalifiany *et al*. 2021).

   b. Particle Size Analysis

      The particle size was measured using a particle size analyzer (PSA). The nanoemulsion samples (1 mL) were dissolved in ultrapure water (19 mL) in a measuring cup. 4 milliliters of the solution were pipetted and placed into a cuvette. Subsequently, a filled cuvette was inserted into the sample holder (Larasati and Jusnita, 2020). The required wavelength is 20–500 nm (Indalifiany *et al*., 2021).

   c. Zeta Potential

      2 grams of the emulsion was mixed with 5 mL of deionized water and the zeta potential was measured using electrophoretic light scattering (Christian *et al*., 2022). Stable nanoemulsion formulations have a high zeta potential (± 30 mV) (Handayani *et al*., 2018).

   d. Nanoemulsion Type Test

      The nanoemulsion was dripped into a methylene blue solution. If the blue color is dispersed throughout the emulsion, it is an O/W emulsion type, but if the blue color is not completely dispersed, it is an O/W emulsion type (Pratasik *et al*., 2019).

5. Nanoemulgel Preparations

   The nutmeg seed oil nanoemulgel was prepared by mixing the nanoemulsion into the gel base in a 1:1 ratio. The nutmeg oil nanoemulgel formulation is shown in Table II, where the mixture of nanoemulsion and gel base was stirred using a mortar and pestle until a homogeneous nanoemulgel was formed (Rehman *et al*., 2022).

Table II. Nutmeg Seed Oil Nanoemulgel Formulation

No	Bahan	Formulasi (gram)
1	Nutmeg Seed Oil Nanoemulsion	100
2	Gliserin	10.4
3	Methyl paraben	0.1
4	Carbopol 940	0.75
5	TEA	Qs
6	Aquadest	Ad 200

6. Evaluation Test of Nanoemulgel
   a. Organoleptic

   Organoleptic testing of nutmeg oil nanoemulgel preparations was performed by observing the color, odor, and texture of the preparations (Hajrah *et al*., 2017). The requirements for good preparation are that it is white and has the characteristic smell of nutmeg oil.

   b. Homogeneity

   Homogeneity testing was performed by smearing a sample of the nanoemulgel preparation. The homogeneous preparation showed no visible coarse grains (Hajrah *et al*., 2017). The requirement for a good homogeneity test is that it should be free from clumped particles (Pratasik *et al*., 2019).

   c. pH Test

   1 gram of the nanoemulsion was diluted with 10 mL of distilled water and the pH of the preparation was measured (Lumentut *et al*., 2020). The requirement for good pH is the pH of the skin (4.5–6.5 (Hajrah *et al*., 2017).

   d. Viscosity Test

   The viscosity of the preparation was measured using a Rion Viscometer, 50 grams of the preparation was put into the pot, using spindle no. 4 and the rotor was turned on. The viscosity results were recorded and replicated 3 times (Pratasik *et al*., 2019). The requirement for good viscosity is 3,000–50,000 cps (Sulastri and Zamzam, 2018).

   e. Spreadability

   0.5 grams of nanoemulgel preparation was weighed and placed in the center of the first petri dish that had been given a millimeter block. The second Petri dish was placed on top of the first Petri dish for one minute. The diameter of the nanoemulgel was measured on the side of the Petri dish, and repeated measurements were performed by adding a load of 50 grams every minute until the load was 300 grams. Testing was performed three times (Imanto *et al*., 2019). The required spreading power is 5–7 cm (Pratasik *et al*., 2019).

   f. Adhesion Test

   The nanoemulsion preparation (0.25 g) was weighed and placed on an object glass, and another object glass was placed on top of the preparation. Subsequently, a load of 1 kg was added for 5 minutes to the object glass and attached to the test equipment. A weight of 80 grams was released, and the time until the two object glasses were released was recorded (Imanto *et al*. 2019). The requirement for good adhesion is more than 4 seconds (Pratasik *et al*., 2019).

   g. Rheology Test

   The rheological properties were measured using a Rion viscometer with a cylindrical spindle to determine the flow properties of the prepared preparations. A total of 300 mL of the preparation was placed in the sample container and the spindle was rotated at speeds of 3, 6, 12, 30, and 60 rpm to obtain a torque value in the range of 3–97% (Dianingsih *et al*., 2016). The emulgel preparation has pseudoplastic flow properties, indicating a non-Newtonian flow type (Priani *et al*., 2021).

7. Freeze-Thaw Stability Test

   Stability testing of the nutmeg oil nanoemulsion preparation was carried out by storing the samples at different temperatures for 6 days. The nanoemulsion was stored at cold temperature (± 4°C) for 24 hours. Subsequently, it was placed at a high temperature (± 40°C). The storage process at low and high temperatures was calculated as 1 cycle (Iradhati and Jufri, 2017).

8. Penetration Test
   a. Determination of Calibration Curve

      Nutmeg oil (100 mg) was weighed and dissolved in a phosphate buffer solution in a 100 mL volumetric flask. A solution with a concentration of 1000 ppm was obtained. Next, solutions were prepared with concentrations of 5, 6, 7, 8, 9, and 10 ppm, and the absorption at maximum λ was measured using a UV-Vis spectrophotometer (Chandra *et al*., 2019).

   b. Determination of Nutmeg Oil Content in Nanoemulgel Preparation

      A total of 1 gram of the preparation was weighed, dissolved in a phosphate buffer solution in a 25 mL volumetric flask, and then filtered through filter paper. Then, 2 mL of the solution was placed into a 10 mL measuring flask, and the volume was made up by adding a phosphate buffer solution of pH 7.4. The test solution was then analyzed with a UV-Vis spectrophotometer at maximum λ (Chandra *et al*., 2019).

   c. Penetration Test on Franz Diffusion Cells

      The in vitro penetration test was carried out using circle-shaped Whatman No. 1 filter paper with an area of 1.76 cm^2. Whatman filter paper no.1 was soaked in buffer for 5 minutes. The penetration medium used 50 mL of phosphate buffer solution (pH 7.4 ± 0.05) and heated over a water bath at a temperature of 37°C ± 0.05°C with stirring at a speed of 500 rpm. Next, 1 g of the nanoemulgel preparation was weighed and spread evenly on filter paper placed in the donor compartment. Next, 5 mL samples were taken at 5, 10, 15, 45, 60, 90, and 120 minutes, changing to a phosphate buffer solution of pH 7.4 ± 0.05 for each sample. Perform analysis on The sample was analyzed using a UV-Vis Spectrophotometer at the maximum λ (Nurwaini and Purbowati, 2019). The penetration speed of the nutmeg oil nanoemulgel through the membrane was calculated using Fick's I Law.

Data Analysis

The observation results were analyzed statistically using the Statistical Package for the Social Sciences (SPSS) software with the ANOVA test, which is a method with a confidence level of 95% (Imanto *et al*., 2019).

RESULTS AND DISCUSSION

1. Characteristics of Nutmeg Oil

   The characteristic test is a process carried out to determine the quality and grade of simplicia or oil so that the test results can be used as a guide for further research (Supomo, 2016). The test results for the characteristics of nutmeg oil showed that it had a slightly yellow color and a characteristic smell of nutmeg oil, a specific gravity used pycnometer of 0.8999 g/mL, a refractive index used refractometer of 1.479, a clear solubility in 90% ethanol 1:3, an optical rotation using a polarimeter of 23.1°, and a residual evaporation used gravimetry of 0.7%. Based on these results, the nutmeg oil used meets the SNI requirements and is based on the certificate of analysis (COA), so it can be used as a material for further research.

2. Results of Evaluation Tests for Nutmeg Oil Nanoemulsion

   The test parameters for nanoemulsion preparations include turbidimetry, particle size analysis, zeta potential tests, pH tests, and emulsion type tests, and the results are shown in Table III.

Table III. Result of Nanoemulsion Evaluation

Test Parameters	F1	F2	F3	Requirements
Turbidimetry	91,1 %	90,7 %	90,7 %	90 – 100 % (Indalifiany *et al*., 2021)
Particle Size Analysis	471 nm	453 nm	516 nm	20 – 500 nm (Pratasik *et al*., 2019)
Zeta Potential	42.5 mV	48.6 mV	42.4 mV	- 30mV to > 30mV (Handayani *et al*., 2018)
pH Test	6.66	6.51	6.46	4.5 – 6.5 (Hajrah *et al*., 2017)
Emulsion Type Test	O/W	O/W	O/W	O/W (Pratasik *et al*., 2019)

The evaluation results in Table III show that the nanoemulsion has an opacity close to that of water, because the transmittance value obtained is in the range of 90–100%. The results of the particle size analysis in F1 and F2 showed good results in the range of 20–500 nm. The particle size of F3 did not meet the requirements because it contained the highest concentration of nutmeg oil. The zeta potential test results show values in the range of- 30mV to > 30mV, with a pH value that meets the requirements of formula 3. Nutmeg oil nanoemulsions have the same emulsion type, namely O/W, although they contain different concentrations of nutmeg oil.

3. Result of Evaluation Tests for Nutmeg Oil Nanoemulgel
   a. Organoleptic

      Organoleptic test results showed that all formulas were white, had a distinctive smell of nutmeg oil, and had a semisolid texture.

   b. Homogeneity

      Based on the observation results, all nanoemulgel formulas had good homogeneity as indicated by the absence of lumpy particles.

   c. pH Test

      The pH measurement results are presented in Table IV. Based on the pH measurements, each nanoemulgel preparation before and after the stability test had a good pH value because the pH value obtained met the requirements (Hajrah *et al*., 2017). The pH value in each formula decreased because of the different nutmeg oil concentrations. A significance value < 0.001 was obtained based on the results of the one-way ANOVA test. This shows that the concentration of nutmeg oil has a significant influence on the changes in pH because a significance value of <0.05 was obtained. Nutmeg oil contains the compounds like myristicin, alpha-pinene, sabinene, and beta-pinene. Nutmeg oil has an acidic pH of 4.99. Thus, the higher the concentration of nutmeg oil, the more acidic is the preparation.

   d. Viscosity Test

      Based on the observation results shown in Table IV, each nanoemulgel preparation before and after the stability test had a good viscosity value because the results obtained met the requirements, namely in the range of 2000–50000 cPs. From these results, it was determined that the viscosity increased as the concentration of nutmeg oil increased. This is because the concentration of a preparation shows a large number of dissolved particles in each volume such that more dissolved particles will produce greater friction between particles with a higher viscosity value (Lumbanturoan and Yulianti, 2016). Based on the results of the statistical analysis of the one-way ANOVA, a significance value of 0.042 was obtained. This shows that the concentration of nutmeg oil has a significant influence on the changes in viscosity because a significance value of <0.05 was obtained.

   e. Spreadability

      The results of the spreadability tests are listed in Table IV. Based on these observations, each nanoemulsion preparation before and after the stability test had good spreadability because the obtained results met the requirements in the range of

5–7 cm (Pratasik *et al*., 2019). The results of measuring The spreadability of nutmeg oil nanoemulgel decreased in each formula, which was due to the different concentrations of nutmeg oil in each formula. Based on the results of the statistical analysis of the one-way ANOVA, a significance value of 0.027 was obtained. This shows that the concentration of nutmeg oil has a significant influence on the changes in spreadability because a significance value of <0.05 was obtained. The spreadability is closely related to the viscosity of the preparation. The smaller the viscosity, the greater the spreadability of the preparation; conversely, as the viscosity value increases, the preparation will be thicker, and thus the diameter of the spreading power will be smaller (Rahmatullah *et al*., 2020).

f. Adhesion Test

Based on the results shown in Table IV, the nanoemulgel preparation in F3 after the stability test did not meet the requirements. This is because the temperature during the stability test can affect changes in adhesive power, the higher the temperature will cause more substances to evaporate in the preparation and will produce a thick preparation (Hendrawan *et al*., 2020). The adhesion value in each formula decreased because of the different nutmeg oil concentrations. Based on the results of the statistical analysis of the one-way ANOVA test, a significance value of 0.037 was obtained. This shows that the concentration of nutmeg oil has a significant influence on the changes in adhesive strength because a significance value of <0.05 was obtained. The greater the concentration of nutmeg seed oil in the nanoemulgel preparation, the lower the adhesive power because the characteristics of the nanoemulgel become oilier (Kurnianto *et al*., 2017). Long adhesion to the skin surface is expected to prolong the contact between the active substance and the skin and will provide a more optimal therapeutic effect.

g. Rheology Test

Based on Figure 1, a nanoemulgel flow type was obtained, including pseudoplastic flow. A pseudo-plastic flow is a type of flow that experiences a decrease in viscosity when the shear speed increases (Dianingsih *et al*., 2016).

Table IV. Result of Evaluation Tests for Nutmeg Oil Nanoemulgel

Formula	Before Freeze-Thaw	After Freeze-Thaw	Requirements
Result of pH Test			
F1	6.37	6.34	4.5 – 6.5
F2	6.27	6.28	(Hajrah *et al*.,
F3	6.14	6.26	2017)
Result of Viscosity Test (cPs)			
F1	7423	6658	2000-50000
F2	8873	8023	(Lumbanturoan
F3	9889	9518	and Yulianti,
			2016)
Result of Spreadability Test (cm)			
F1	6.1	6.1	5.0-7.0
F2	6.0	5.7	(Pratasik *et al*.,
F3	5.8	5.3	2019)
Result of Adhesion Test (s)			
F1	8.22	5.27	> 4
F2	6.20	4.17	(Hendrawan *et*
F3	5.30	3.16	*al*., 2020)

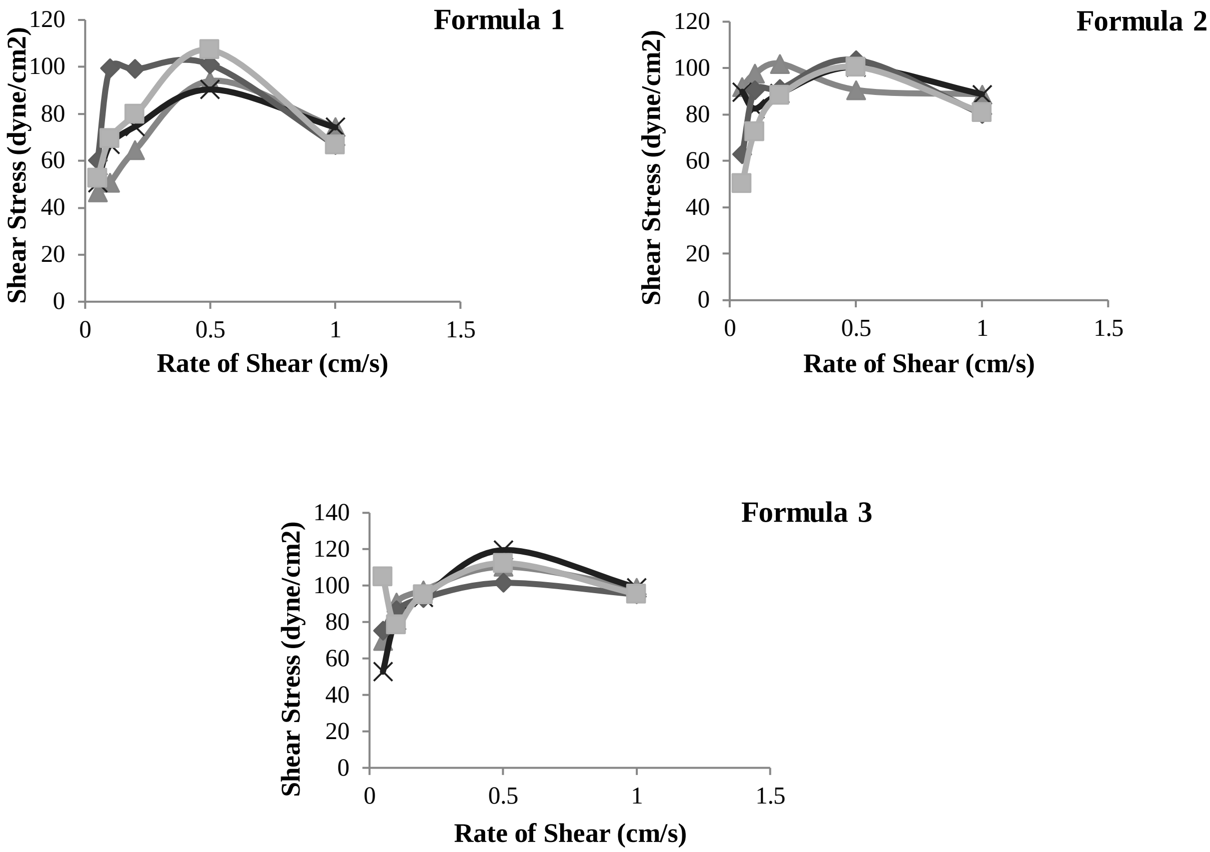

Figure 1. Result of Rheology Test

4. Result of Penetration Test

Penetration testing is a process carried out to determine the amount of substance that penetrates the skin during a certain time interval (Andini *et al.*, 2016). The penetration test method used was the Franz Diffusion Cell with the working principle of placing a semi-permeable membrane between the components of the donor and receptor compartments, after which the compounds penetrated the membrane layer into the solution in the receptor compartment. Compound levels were measured quantitatively using UV-Vis spectrophotometric analysis (Chandra *et al.*, 2019).

After obtaining the absorbance measurement results for each sample, the cumulative amount of substance penetrated, percentage of the cumulative amount penetrated, and penetration speed for each formula were calculated for each sample. The results of the graph of the cumulative amount of active substances penetrated are shown in Figure 2.

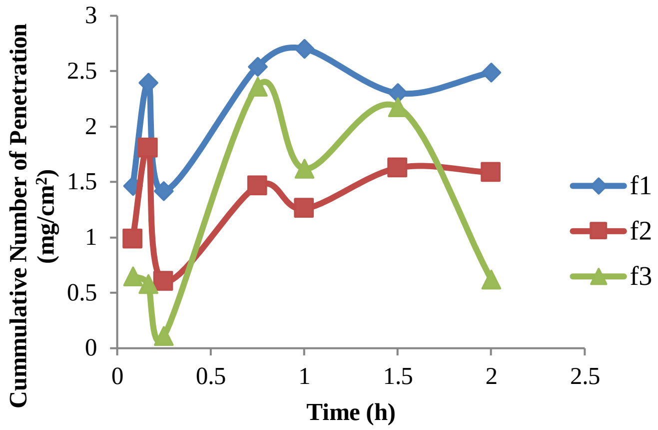

Figure 2. Result of Cumulative Number of Penetration

The cumulative amount of penetration was plotted against time, and a linear regression equation was created to obtain the penetration flux for each preparation. The results obtained from the penetrating flux in each formula were F1 0.0117 mg/cm^2hour^{-1}, F2 0.0126 mg/cm^2hour^{-1}, and F3 0.0246 mg/cm^2hour^{-1}. Based on these results, the highest flux value was obtained with formula F3 because it had the highest concentration of nutmeg oil. This indicated that F3 had the fastest penetration speed. According to (Laelatussilmi, 2018), the greater the drug concentration, the faster the diffusion speed. The penetration speed measurements were performed based on Ficks' Law 1. According to Ficks' Law 1, the penetration of a compound is directly proportional to the cumulative amount of the active substance that can influence the penetration flux (Fahruzzaman, 2017). The graph of the flux measurement results is shown in Figure 3.

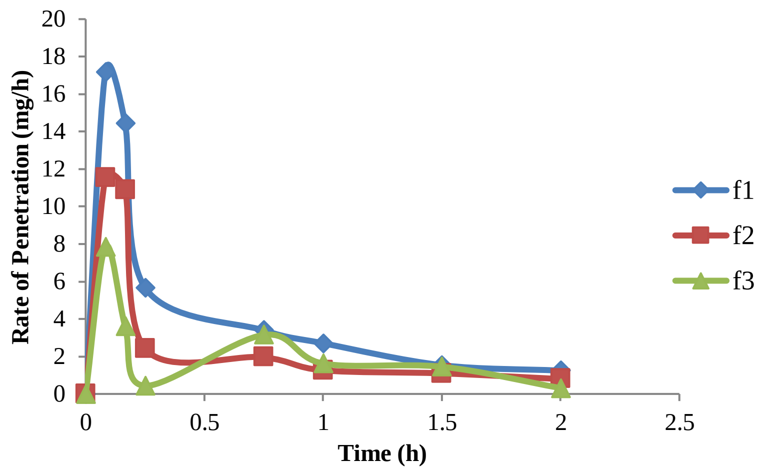

Figure 3. Penetration Flux

Penetration flux is used to determine the ability of a substance to penetrate (Mulyana *et al*., 2016). Based on the penetration rate results (Figure 3), a spike occurred at 5 minutes indicating that the active substance started to penetrate the membrane. In the following minutes, the active substance that has penetrated will continue to enter until it reaches a steady state, which is caused by the large difference in concentration gradient between the donor and receptor compartments. The steady-state condition is reached at 60 minutes, which is marked by the rate of drug penetration becoming constant (Mulyana *et al*., 2016).

Based on these results, it was found that the penetration flux decreased for each formula. This is due to the influence of particle size on each concentration of nutmeg oil, which is in line with previous research that states that the smaller the particle size, the faster is the penetration into the skin. In this study, nanoemulsion formulations used Tween 80 at a concentration of 45% as a nonionic surfactant. Nonionic surfactants are not electrically charged, and their binding to proteins is limited or absent owing to weak hydrophobic interactions, which do not cause protein denaturation. Additionally, nonionic surfactants can penetrate the stratum corneum more easily than anionic surfactants, which strongly bind to the stratum corneum and tend to cause less irritation to the skin (Yuan *et al*., 2014).

Statistical analysis was performed by performing a normality test to determine whether the data were normally distributed, as indicated by a significance value > 0.05. Based on the results of statistical analysis, the data were not normally distributed because the significance value was <0.05. Therefore, data analysis was continued using the non-parametric test, namely Kruskal Wallis, and a significance value of 0.350 was obtained. This shows that the concentration of nutmeg oil did not have a significant influence on the penetration rate because the significance value obtained was > 0.05. This may be because the active substance that has been mixed in the preparation has a change in particle size, which can affect the penetration rate.

CONCLUSION

Based on these results, variations in the concentration of nutmeg oil (*Myristica fragrans* Houtt.) can influence the physical characteristics of the nanoemulgel preparations, such as pH, spreadability, viscosity, and rheology, and obtained good stability. Variations in the nutmeg oil concentration did not affect the penetration rate of the nanoemulsion preparation in vitro.

REFERENCES

Agustina, R., Tita Indrawati, D., and Amir Masruhin, M, 2015, Aktivitas Ekstrak Daun Salam (*Eugenia polyantha*) Sebagai Antiinflamasi Pada Tikus Putih (*Rattus Norvegicus*). *J. Trop. Pharm. Chem. 3*(2).

Andini, S., Jufri, M., and Djajadisastra, J, 2016, Formulasi dan Uji Penetrasi Sediaan Gel Transfersom yang Mengandung Kojyl 3 Aminopropyl Fosfat sebagai Pencerah Kulit, *Jurnal Kefarmasian Indonesia. 6*(2). 129–136.

Chandra, D, 2019, Pengujian Penetrasi In-Vitro Sediaan Gel, Krim, Gel-Krim Ekstrak Biji Kopi (*Coffea arabica* L.) Sebagai Antiselulit, *Jurnal Ilmiah Farmasi Imelda. 3*(1).

Christian, Y. E., Rahmat, D., and Farida, Y, 2022, Formulasi Nanoemulgel Ekstrak Daun Cantigi (*Vaccinium varingiaefolium* Miq.) sebagai Antioksidan. *Majalah Farmasetika*, *7*(5), 478–493.

Damayanti, H., Wikarsa, S., and Jafar, G, 2019, Formulasi Nanoemulgel Ekstrak Kulit Manggis (*Garcinia mangostana* L.), *Jurnal Riset Kefarmasian Indonesia. 1*(3), 166–176.

Dhawas, V., Dhabarde, D., and Patil, S, 2020, Emulgel : A Comprehensive Review for Novel Topical Drug Delivery System, *International Journal of Recent Scientific Research. 11*(04), 38134–38138. https://doi.org/10.24327/ijrsr.2020.1104.5247

Dianingsih, N., Hari Purnomo, E., and R. Muchtadi, T, 2016, Sifat Reologi dan Stabilitas Fisik Minuman Emulsi Minyak Sawit. *Jurnal Teknologi dan Industri Pangan*. *27*(2), 165–174. https://doi.org/10.6066/jtip.2016.27.2.165

Eid, A. M., El-Enshasy, H. A., Aziz, R., and Elmarzugi, N. A, 2014, Preparation, Characterization and Anti-Inflammatory Activity of *Swietenia macrophylla* Nanoemulgel. *Journal of Nanomedicine and Nanotechnology*, *5*(2). https://doi.org/10.4172/2157-7439.1000190

Fahruzzaman, A, 2017, Uji Penetrasi Gel Transdermal Nanopartikel Glukosamin Hidroklorida dengan Variasi Konsentrasi Kitosan menggunakan Sel Difusi Franz. *Skripsi*. UIN Syarif Hidayatullah.

Fitra Suloi, A., Nur, A., and Suloi, F, 2021, Bioaktivitas Pala (*Myristica fragrans* Houtt) : Ulasan Ilmiah. *Jurnal Teknologi Pengolahan Pertanian*, *3*(1), 11–18.

Hajrah, Meylina, L., Sulistiarini, R., and Puspitasari, L, 2017, Optimasi Formula Nanoemulgel Ekstrak Daun Pidada Merah (*Sonneratia caseolaris* L.) dengan Variasi *Gelling Agent*, *Jurnal Sains dan Kesehatan*. *1*(7).

Handayani, F. S., Nugroho, B. H., and Munawiroh, S. Z, 2018, Optimization of Low Energy Nanoemulsion of Grape Seed Oil Formulation Using D-Optimal Mixture Design (DMD). *Jurnal Ilmiah Farmasi*, *14*(1), 17–34. http://journal.uii.ac.id/index.php/JIF

Hendrawan, I., Suhendra, L., and Ganda Putra, G, 2020, Pengaruh Perbandingan Minyak dan Surfaktan serta Suhu terhadap Karakteristik Sediaan Krim, *Jurnal Rekayasa dan Manajemen Agroindustri*. *8*(4).

Ikhtiyarini, T. A. and Sari, A. K, 2022, Efektivitas Penggunaan Basis Gel pada Sediaan Emulgel, *Camellia*. *1*(1), 19–25.

Imanto, T., Prasetiawan, R., and Retno Wikantyasning, E, 2019, Formulation and Characterization of Nanoemulgel Containing *Aloe vera* L. Powder, *Jurnal Farmasi Indonesia*. *16*(1). http://journals.ums.ac.id/index.php/pharmacon

Indalifiany, A., Malaka, M. H., Sahidin, Fristiohady, A., and Andriani, R, 2021, Formulasi dan Uji Stabilitas Fisik Nanoemulgel Ekstrak Etanol Spons *Petrosia Sp*, *JFSP*. *7*(3), 321–331.

Iradhati, A. H. and Jufri, M, 2017, Formulation and Physical Stability Test of Griseofulvin Microemulsion Gel, *International Journal of Applied Pharmaceutics*. *9*, 23–26. https://doi.org/10.22159/ijap.2017.v9s1.22_27

Keservani, R. K. and Sharma, A. K. 2019. *Nanodispersions for Drug Delivery*. Apple Academic Press. New York.

Kim, D. M., Hyun, S. S., Yun, P., Lee, C. H., & Byun, S. Y, 2012, Identification of an Emulsifier and Conditions for Preparing Stable Nanoemulsions Containing the Antioxidant Astaxanthin. *International Journal of Cosmetic Science*, *34*(1), 64–73. https://doi.org/10.1111/j.1468-2494.2011.00682.x

Kurnianto, E., Sugihartini, N., and Nurani, L. H, 2017, Hubungan Antara Konsentrasi Minyak Atsiri Kayu Manis (*Cinnamomum burmannii* Nees Ex Bl.) dalam Lotion dengan Sifat Fisik dan Tingkat Kesukaan Konsumen, *Alaba: Jurnal Litbang Pengendalian Penyakit Bersumber Binatang Banjarnegara*. *13*, 21–28. https://doi.org/10.22435/blb.V13i1

Laelatussilmi, F. 2018. Pengaruh Penambahan Tween 80 Sebagai Enhancer Terhadap Sifat Fisik dan Difusi Sediaan Gel Natrium Diklofenak Secara *In Vitro*. *Skripsi*. Universitas Muhammadiyah Purwokerto.

Larasati, S. P. and Jusnita, N, 2020, Nanoemulsion Formulation of Turmeric Extract (*Curcuma longa* L.) as An Antioxidant. *Journal of Pharmaceutical and Sciences*, 33–41.

Lumbanturoan, P. and Yulianti, E, 2016, Pengaruh Suhu terhadap Viskositas Minyak Pelumas (oli), *Sainmatika*. *2*(13), 26–34.

Lumentut, N., Edy, E. M., and Rumondor, E. M, 2020, Formulasi dan Uji Stabilitas Fisik Sediaan Krim Ekstrak Etanol Kulit Buah Pisang Goroho (*Musa acuminafe* L.) Konsentrasi 12.5% Sebagai Tabir Surya, *Jurnal MIPA*. *9*, 42–46. https://doi.org/https://doi.org/10.35799/jmuo.9.2.2020.28248

Matulyte, I., Jekabsone, A., Jankauskaite, L., Zavistanaviciute, P., Sakiene, V., Bartkiene, E., Ruzauskas, M., Kopustinskiene, D. M., Santini, A., & Bernatoniene, J, 2020, The Essential Oil and Hydrolats from *Myristica fragrans* Seeds with Magnesium Aluminometasilicate as Excipient: Antioxidant, Antibacterial, and Anti-inflammatory Activity, *Foods*. *9*(1). https://doi.org/10.3390/foods9010037

Mulyana, S., Fahrurroji, A., and Riza, H, 2016, Pengaruh Propilen Glikol Terhadap Penetrasi Gel Hesperidin Secara In Vitro, *Jurnal Mahasiswa Farmasi Fakultas Kedokteran UNTAN.*

Nurwaini, S. and Purbowati, A. 2019, Kombinasi Asam Oleat dan Propilen Glikol sebagai *Enhancer Cold Cream* Verapamil untuk Penghantaran Transdermal, *University Research Colloqium.*

Pratasik, M. C., Yamlean, P. V., and Wiyono, W. I, 2019, Formulasi dan Uji Stabilitas Fisik Sediaan Krim Ekstrak Etanol Daun Sesewanua (*Clerodendron squamatum* Vahl.), *PHARMACON*. *8*(2).

Priani, S. E., Anggara Permana, R., Nurseha, M., and Aryani, R, 2021, Pengembangan Sediaan Emulgel Antioksidan dan Tabir Surya Mengandung Ekstrak Kulit Buah Cokelat (*Theobroma cacao* L). *Jurnal Farmasi Dan Ilmu Kefarmasian Indonesia*, *8*(3), 264.

Rahmatullah, S., Ningrum, W. A., and Dewi, N. K, 2020, Formulasi dan Evaluasi Sediaan Gel *Hand Sanitizer* Sebagai Antiseptik Tangan dengan Variasi Basis Karbopol 940 dan TEA. *Pharmaceutical Scientific Journal*, *3*, 189–194.

Rehman, A., Iqbal, M., Khan, B. A., Khan, M. K., Huwaimel, B., Alshehri, S., Alamri, A. H., Alzhrani, R. M., Bukhary, D. M., Safhi, A. Y., and Hosny, K. M, 2022, Fabrication, In Vitro, and In Vivo Assessment of Eucalyptol-Loaded Nanoemulgel as a Novel Paradigm for Wound Healing. *Pharmaceutics*, *14*(9).

Shabrina, A., Safitri, E. I., and Pratiwi, I, 2021, Stabilitas Fisik dan Antioksidan Mikroemulsi Minyak Biji Pala dengan Variasi Tween 80 – PEG 400, *Media Farmasi*. *17*(1), 25. https://doi.org/10.32382/mf.v17i1.2049

Sulastri, L. and Zamzam, M. Y, 2018, The Formulation Gel of Hand Sanitizer of Basil Leaves Ethanol Extract Concentrations of 1,5%, 3%,and 6% with Gelling agent Carbopol 940, *Medimuh. 1*(1), 31–43.

Supomo, Supriningrum, R., and Junaid, R, 2016, Karakteristik dan Skrining Fitokimia Daun Kerehau (*Callicarpa longifolia* Lamk.). *Jurnal Kimia Mulawarman*, *2*(13), 89–96.

Yuan, C. L., Xu, Z. Z., Fan, M. X., Liu, H. Y., Xie, Y. H., and Zhu, T, 2014, Study on Characteristics and Harm of Surfactants, *Journal of Chemical and Pharmaceutical Research*. *6*(7), 2233–2237.