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Research Article

Physical Stability and Effectivity Comparison of Nanoemulsion and Nano-emulgel of Avocado Oil with Carbopol 940 Variation

Ayu Shabrina[1*], Junvidya Heroweti[2], Gharsina G. Yumni[3], Agnes T. Poerba[4], Diana A. Setyani[4]

[1]Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmacy, Universitas Wahid Hasyim, Semarang, Jawa Tengah

[2]Department of Pharmacology and Clinical Pharmacy, Faculty of Pharmacy, Universitas Wahid Hasyim, Semarang, Jawa Tengah

[3]Department of Chemical Pharmacy, Faculty of Pharmacy, Universitas Wahid Hasyim, Semarang, Jawa Tengah

[4]Faculty of Pharmacy, Universitas Wahid Hasyim, Semarang, Jawa Tengah

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*Corresponding author: shabrina@unwahas.ac.id

Abstract

Avocado oil (AV) contains of oleic acid that can prevent skin erythema. Nanoemulsion (NE) in a gel system (NG) can increases the drug's effectivity. This study aimed to compare AV's stability and effectivity as anti-erythema in NE and NG with variations of carbopol 940. NE was made with 5% of AV and incorporated in carbopol 940 (for NG) with variations of 0.5 (F1);1 (F2), and 1.5% (F3). The samples were stored in a climatic chamber for 90 days at a temperature of 30 °C ± 2 °C RH 65% ± 5%. Samples were tested for organoleptic, pH, viscosity, particle size (PS), polydispersity index (PDI), and zeta potential (ZP). The samples were tested for in vitro SPF values and minimum erythema dose (MED). F2 and F3 of NG showed a good physical characteristic. NE met the requirements of nanoemulsion delivery. Both NE and NG stable for 90 days of storage. The best SPF value was NE at 20.22 ± 2.21 and MED of 221.5 ± 7.33 minutes. NG F1 of 16.84 ± 2.30 with MED of 225.2 ±4.42 minutes. Carbopol at a concentration of 0.5-1.5% can produce stable NG. Avocado oil nanoemulsion showed better effectivity to prevent erythema than nano-emulgel.

Keywords: Avocado oil, Erythema, Nanoemulsion, Nano-emulgel, Stability.

Perbandingan Stabilitas Fisik dan Efektivitas Nanoemulsi dan Nano-emulgel Minyak Alpukat dengan Variasi Carbopol 940

Abstrak

Minyak alpukat (AV) mengandung asam oleat yang dapat mencegah eritema kulit. Nanoemulsi (NE) dalam sistem gel (NG) dapat meningkatkan efektivitas zat aktif. Penelitian ini bertujuan untuk membandingkan stabilitas dan efektifitas AV sebagai anti eritema pada NE dan NG dengan variasi carbopol 940. NE dibuat dengan AV 5% dan dimasukkan ke dalam carbopol 940 (untuk NG) dengan variasi 0.5 (F1);1 (F2), dan 1,5% (F3). Sampel disimpan dalam *climatic chamber* selama 90 hari pada suhu 30 °C ± 2 °C RH 65% ± 5%. Sampel diuji organoleptik, pH, viskositas, ukuran partikel (PS), indeks polidispersitas (PDI), dan potensial zeta (ZP). Sampel diuji nilai SPF in vitro dan *Minimum Erythema Dose* (MED). F2 dan F3 pada NG menunjukkan karakteristik fisik yang baik. NE memenuhi persyaratan penghantaran nanoemulsi. Baik NE maupun NG stabil selama penyimpanan 90 hari. Nilai SPF terbaik adalah NE sebesar 20,22±2,21 dan MED sebesar 221,5±7,33 menit. Nilai SPF NG F1 sebesar 16,84 ± 2,30 dengan MED 225,2 ±4,42 menit. Karbopol pada konsentrasi 0,5-1,5% dapat menghasilkan NG yang stabil. Nanoemulsi minyak alpukat menunjukkan efektivitas yang lebih baik dalam mencegah eritema dibandingkan nano-emulgel.

Kata Kunci: Eritema, Minyak alpukat, Nanoemulgel, Nanoemulsi, Stabilitas

1. Introduction

Excessive sun exposure can cause sunburn, photoaging and dry skin.[1] Treating dry skin from sunburn can be done using moisturizer and sunscreen.[2] The benzyl benzoate, benzyl cinnamate and benzyl salicylate in moisturizer and sunscreen products can cause allergies and irritation to the skin.[3] The FDA states that long-term use of these allergenic components can cause reproductive disorders and possibly cause cancer.[4] It is necessary to develop products made from natural ingredients that can treat sunburn and moisturize the skin.

Avocado oil is a natural ingredient that can be used as a moisturizer component.[5] Avocado oil contains oleic, linoleic, and linolenic acid, which moisturize the skin by preventing water loss.[6] Previous studies have shown that using avocado oil directly can moisturize the skin, prevent wrinkles, provide an antibacterial effect and heal scars.[7,8] Using avocado oil directly may give the impression of being oily and sticky. Many researches showed that avocado oil had been used in nanoemulsion (NE) as carrier oil.[9] Microemulsion of avocado oil as active substance had been done for treating wound healing yet the content of the active substance was 50%, so it is considered ineffective as a cosmetic.[10]

Avocado oil are mostly packed in opaque bottles and used for carrier oil. This packaging is effectively reducing light exposure to increase the stability. Other than tackling these issues through package design, attempts to formulate the oils minimized the exposure and losses.[11] For this reason, an effective formula and delivery system for avocado oil is necessary.

The poor stability and permeability of avocado oil into/through the skin has limited its clinical applications. Nano-emulgel (NG) is a capable delivery system for lipophilic substances that supports the permeation of active substances into the skin.[11] Nano-emulgel is made by incorporating a nanoemulsion system into a gel base. The gel base is essential in this delivery system.[12] Nano-emulgel with carbopol 940 can increase the effectiveness of the active substance.[13] Carbopol 940 has been used in several nano-emulgel research, which supports the effectivity and stability of the active substance as an anti-inflammatory, anti-acne and wound healing.[14]

To our knowledge, the current study is the first to report the use of nanoemulsion (NE) and nano-emulgel (NG) into appropriate gel base such as Carbopol 940. In addition, in vitro and ex vivo sunscreen effectivity were carried out compared with a marketed sunscreen gel.

2. Method

2.1. Tools

The tools used in the research were a pH meter (Electrolab®, England), a viscometer (Rheosys Merlyn II®, Germany), a UV spectrophotometer (Shimadzu®, China), a particle size analyzer and a climatic chamber (HWS-70 BX®, China), magnetic stirrer (SCILOGEX MS-H280 Pro®, Germany), particle size analyzer (SZ-100®, China).

2.2. Materials

The material used in this research is avocado oil obtained from PT Daarjeling Aroma Bandung with cosmetics grade and equipped with a certificate of analysis. Other ingredients were Tween 80 (Chemsino®), PEG 400(Chemsino®), benzyl alcohol(Chemsino®), span 80(Chemsino®), sorbitol(Chemsino®), paraffin liquid (Lansida®), carbopol 940 (Aqupec®) and TEA (Unichem®) with cosmetics grade. All materials were purchased from Multi Kimia Raya Semarang, Indonesia. The solvent used was distilled water and methanol (Merck®) for analysis with analytical grade specifications obtained from PT Kairos, Yogyakarta, Indonesia.

The test animals were six male rabbits (*Orcyctolagus cuniculus*) with 1,5 to 2 kg weight, healthy and acclimatized. The test animals were obtained from Faculty of Veterinary, Gadjah Mada University. This research had an approval of ethical clearance with number 460/XI/2022/Bioethics Commission published by the Faculty of Medicine and Health Sciences, Universitas

Islam Sultan Agung, Semarang.

2.3. Methods

2.3.1. Avocado oil nanoemulsion and nano-emulgel formulation

The avocado oil nanoemulsion and nano-emulgel formula refer to Table I. Nanoemulsion was made first by dispersing Tween 80 and PEG 400 at a temperature of 50° as mixture 1. Benzil alcohol was then added to the mixture 1. Avocado oil was dropped into the dispersion of mixture 1. Nanoemulgel was made by dissolving Carbopol 940 into hot water at 80°C and then added with TEA. Paraffin liquid, Span 80 and sorbitol were then added to the gel base. Nanoemulsion was incorporated into the emulgel system until homogeneous.[16]

The nano-emulgel and nanoemulsion was stored in a climatic chamber at a temperature of 30 °C ± 2 °C RH 65% ± 5% for stability testing for 90 days. Sampling was carried out before storage (BS), 30th day (D30) and 90th day (D90). Test parameters include organoleptic, homogeneity, pH, viscosity, particle size, zeta potential, polydispersity index, SPF value in vitro and minimum erythema dose (MED).

2.3.2. Homogeneity test

The test was carried out using 5 grams of sample, which was analyzed with 2 glass objects to observe the presence of coarse grains or non-dispersed particles. Another observation made was the phase separation of the nano-emulgel system.[17]

2.3.3. pH Test

Testing was carried out with 10 gram samples. The electrode of the calibrated pH meter is then dipped into the sample and observed until a fixed number appears.[17]

2.3.4. Viscosity Test

Viscosity tests were carried out with a viscometer using 5 grams of sample using rotor no. 2 at 1-60 rpm speed.[18]

2.3.5. Test particle size, zeta potential and polydispersity index

Particle size tests were carried out on the nanoemulsion system and nano-emulgel. Testing was carried out with a particle size analyzer using 10 ml of sample.[15]

2.3.6. In vitro SPF value test

The test was carried out using a UV-vis spectrophotometer at a wavelength of 290-320 nm with intervals of 5 nm. The correction factor used is 10. The absorbance results were calculated using the Mansur method and then grouped into protection categories.[20]

2.3.7. Minimum Erythema Dose (MED) Test

The back of the rabbits was depilated 24 hours before treatment using a shaver. The back skin of animals was divided into five sections for treatment: F1, F2, F3, positive control (Parasol SPF 25++), and negative control (normal skin without UV-Rays exposure). The other section used another rabbit skin, divided into: untreated skin with UV ray exposure and nano-emulsion treatment. Each section was marked with a size of 4x4 cm. The animals were exposed to 311 nm UV light. The number of red spots (erythema) appearing in each test animal group was counted. The time was measured from the beginning of UV light exposure until the erythema appeared. This measurement was calculated as the Minimum Erythema Dose (MED). The MED was calculated by comparing the exposure time of unprotected skin (negative control) with protected skin in every section (F1, F2, F3, and positive control).[21]

2.3.8. Data analysis

Physical characteristics data were analyzed using two-way ANOVA. Data of SPF and MED values from each formula of nanoemulsion and nano-emulgel were analyzed using one-way ANOVA. The effectivity comparison of nanoemulsion and nano-emulgel was analyzed using independent t-test by comparing the SPF value from in vitro test and MED.

3. Result

The result of avocado oil NE and NG

(F1, F2 and F3) can be seen in Figure 1. The result of avocado oil physical stability in NE and NG can be seen in table 1 and 2. The comparison of avocado oil sunscreen effectivity in nanoemulsion and nano-emulgel can be seen in table 3 and Figure 2.

4. Discussion

The avocado oil nanoemulsion was yellowish, transparent and clear with a distinctive aroma, while the avocado oil nano-emulgel was white, yellowish and homogenous. There was no separation of both NE and NG after 90 days of storage. The nanoemulsion's consistency was slightly oily, while the nano-emulgel was watery with no white cast after being applied on the skin. This result is in accordance with previous research, which stated that the appearance of nanoemulsion with Tween 80 and PEG 400 formed a transparent system. In contrast, the gel with a carbopol base formed a homogeneous system and no separation occurred 16,22. Combining tween 80 and PEG 400 can form a clear system with a transmittance percentage of up to 98%.[23] The physical stability data of avocado oil in NE and NG can be seen in table 1 and 2.

The results showed that all formula had a stable pH and did not experience significant changes (p= 0.722) during 90 days of storage. The pH test results met the requirement of SNI (Standar Nasional Indonesia) that topical preparations should have a pH of 4.5-7.2.[24] The results showed that carbopol 940 did not affect the pH during storage. This finding occurs because emulgel formation is influenced by the ratio of carbopol and TEA used.[25] Carbopol added with hydrophilic solvents such as PEG 400 and glycerol can form a good and stable dispersion during storage.[26]

The viscosity test results showed significant differences between nanoemulsion and nano-emulgel in all formula. Nanoemulsion has a lower viscosity due to its liquid consistency. Nano-emulgel F1, F2 and F3 showed differences in viscosity between formula. F3 has the highest viscosity compared to F1 and F2 (p = 0.001). There is a correlation of the concentration of carbopol 940 with an increase of gel viscosity.[27] The higher concentration of carbopol 940 will increase the viscosity o the preparation.[28]

The stability test results showed that the nanoemulsion was stable during 90 days of storage (p = 0.1000). Nano-emulgel in F1 showed a significant increase in viscosity on the 90th day and was significantly different from the viscosity data before the storage and on the 30th day. This is due to aggregation in the nanoemulsion system, which cannot be absorbed by the polymer matrix of the gel base.[29] This finding can be inhibited by increasing the viscosity of the gel base or by increasing the concentration of the base or dissolving the carbopol in propylene glycol or glycerol.[29]

The results of this study showed that nanoemulsion has a particle size below 100

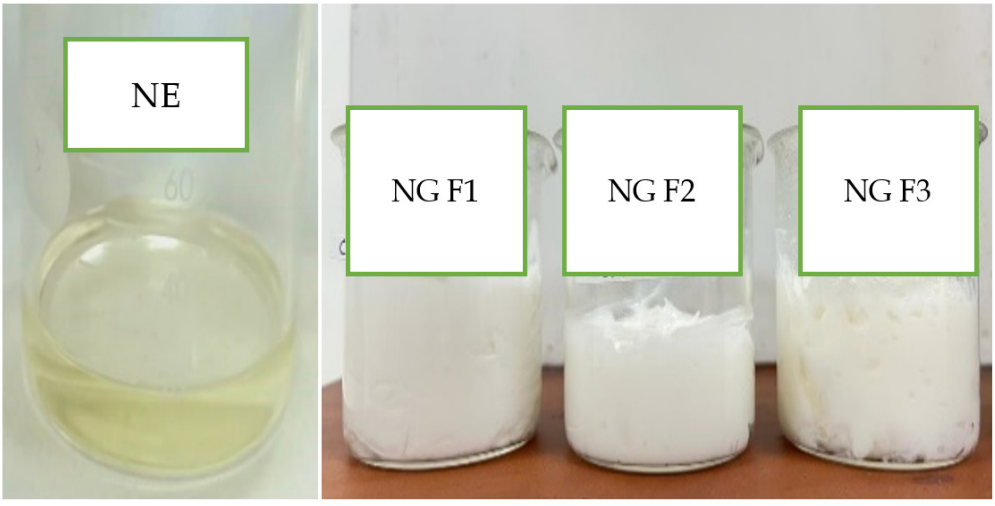

Figure 1. The result of avocado oil NE and NG

Table 1. The stability result of pH and viscosity of avocado oil NE and NG (n=3)

Formula	pH			Viscosity (dPas)		
	BS	D30	D90	BS	D30	D90
NE	6.60±0.1	6.62±0.2	6.61±0.2	80.23±2.55[#]	80.43±1.45[#]	80.76±2.51[#]
NG F1	6.62±0.2	6.63±0.1	6.62±0.2	220.32±5,78a[#]	223.32±2,51 [a#]	320.32±5,78 [a*#]
NG F2	6.63±0.2	6.64±0.4	6.63±0.3	270.25±4,32 [a#]	270.25±4,32 [a#]	270.25±4,32 [a#]
NG F3	6.60±0.0	6.60±0.1	6.62±0.1	320.44±5,61 [a#]	320.44±5,61 [a#]	320.44±5,61[a#]

BS= Before storage; D30= Day 30 of storage; D90= Day 90 of storage
Data displayed with 3 times replication and standard deviation
a) Significantly different compared to nanoemulsion
#Significantly different between different groups in the same column
***Significantly different compared to BS and D30**

nm while the particle size of nano-emulgel was below 300 nm. All formula was included in the nano-sized delivery system category. The results of nano-emulgel particle size were significantly different from nanoemulsion ($p = 0.001$) caused by the aggregation of globules with bases and other solvents in the emulgel.[12] The mixing speed was also one of the factors that causes the particle size of nano-emulgel to be higher than nanoemulsion.[30] Carbopol can experience entrapment in the nanoemulsion system so that the particle size becomes larger compared to the nanoemulsion.[30]

Tween 80 and PEG 400 can form clear and stable nanoemulsions that similar to a micellar system.[31] Tween 80 is a hydrophilic surfactant that can reduce surface tension in nanoemulsion preparations.[9] Carbopol 940 at concentrations of 1% and 1.5% showed good stability. This result showed no significant changes in 90 days of storage ($p = 0.078$). F1 nano-emulgel showed an increase in particle size during 90 days of storage. This indicates particle aggregation during storage, which aligns with increased viscosity.[32] Particle aggregation in the system can influence other parameters, such as the polydispersity index and zeta potential.[33]

All samples showed a PDI value < 1, which indicates that the system is monodisperse.[34] The PDI results showed that the nanoemulsion was monodisperse and did not experience significant changes during 90 days of storage ($p = 0.815$). This monodisperse system was also shown in nano-emulgel, but in F1, on day 30 and day 90 of storage, showed a significant change (0.0039). This is in line with changes in viscosity and particle size so that aggregate formation is possible. Previous research showed that nanoemulsions dispersed into hydrogels produce a polydispersity index of

Table 2. Data of particle size, polydispersity index and zeta potential stability of avocado oil nano-emulgel (n=3)

Formula	Particle Size (nm)			Polydispersity Index			Zeta Potential (mV)		
	BS	D30	D90	BS	D30	D90	BS	D30	D90
NE	16.40 ±2.11	18.40 ±3.18	20.40 ±1.11	0.3± 0.01	0.3± 0.01	0.3 ± 0.01	28.4± 0.01	29.2± 0.00	29.1± 0.01
NG F1	211.43 ±3.24 a	225.43 ±4.15 a	270.05 ±7.45a *	0.4± 0.02 a	0.4± 0.02a	0.5 ± 0.01a*	-27.3± 0.01	-24.2± 0.02	-22.5± 0.00*
NG F2	198.41 ±3.45 a	205.31 ±3.75 a	210.38 ±3.87 a	0.4± 0.03 a	0.4± 0.02a	0.4 ± 0.03 a	-28.3± 0.00	-28.4± 0.00	-29.7± 0.01
NG F3	205.75 ±2,15 a	201.22 ±2.31 a	198,76 ±3,45 a	0.4± 0.02 a	0.4± 0.01a	0.4 ± 0.01 a	-28.5± 0.00	-29.2± 0.01	-29.1± 0.01

BS= Before storage; D30= Day 30 of storage; D90= Day 90 of storage
Data displayed with 3 times replication and standard deviation
***) significantly different compared to D30 and BS**
a) significantly different compared to nanoemulsion

Table 3. Sunscreen Effectivity of nanoemulsion and nano-emulgel of avocado oil

Formula	MED (minutes)	SPF based on MED	SPF Value in vitro
Unprotected skin	13.5 ± 7.41	-	-
NE	221.5 ± 7.33*#	16.41±2.25	20.22 ± 2.21*
NG F1	225.2 ±4.42*#	16.70±1.48	16.84 ± 2.30a*
NG F2	204.4 ±6.45*#	15.17±1.22	15.70 ± 1.25 a*
NG F3	198.8 ± 7.38*#	14.72±2,41	14.68 ± 2.20 a*
Positive control (Parasol)	328.2 ± 4.50*#	24.35±3.25*	20.31 ± 1.30*

n = Data displayed with 3 replications ± standard deviation
***) Significantly different compared to each other group in the same column**
#) Significantly different compared to negative control
a) Significantly different compared to NE and positive control

<0.5 remained stable until 90 days of storage.[35] The PDI of nanoemulsion and nano-emulgel did not differ significantly between formula before or after 30 days of storage (p = 0.811). The increase in PDI during storage was shown to be due to the carbopol gel trapping the nano-droplets into the polymer matrix.[36]

Test results showed that all formula have a zeta potential close to 30 mV which this preparation will stay stable in long term of storage.[12] Zeta potential in all nano-emulgel formula showed negative results influenced by the carboxylate group of carbopol.[37] Carboxylic groups can interact electrostatically when avocado oil droplets are incorporated into the base to obtain a stable colloidal system.[38] Previous research showed that nanoemulsion with a zeta potential of 22.5 mV remained stable during 90 days of storage.[39] The lowest zeta potential value was in F1 and experienced a significant change on day 90, 22.5 mV. This is in line with changes in viscosity and increasing particle size so that the sample is indicated to experience aggregation during storage. Zeta potential results that are close to 0 indicate that the preparation is only stable in the short term.[40] These findings prove that any positive or negative charge shift in the globule system of colloidal particle components will affect the rapid aggregation.[41]

The result of in vitro SPF value and MED can be seen in table 3 and figure 2. The results of in vitro SPF value of nanoemulsion showed a significantly higher value than nano-emulgel (p = 0.0001). All nano-emulgel formula showed no significant differences in SPF values in vitro. This indicates that the release of active substances from the nanoemulsion system is greater than that

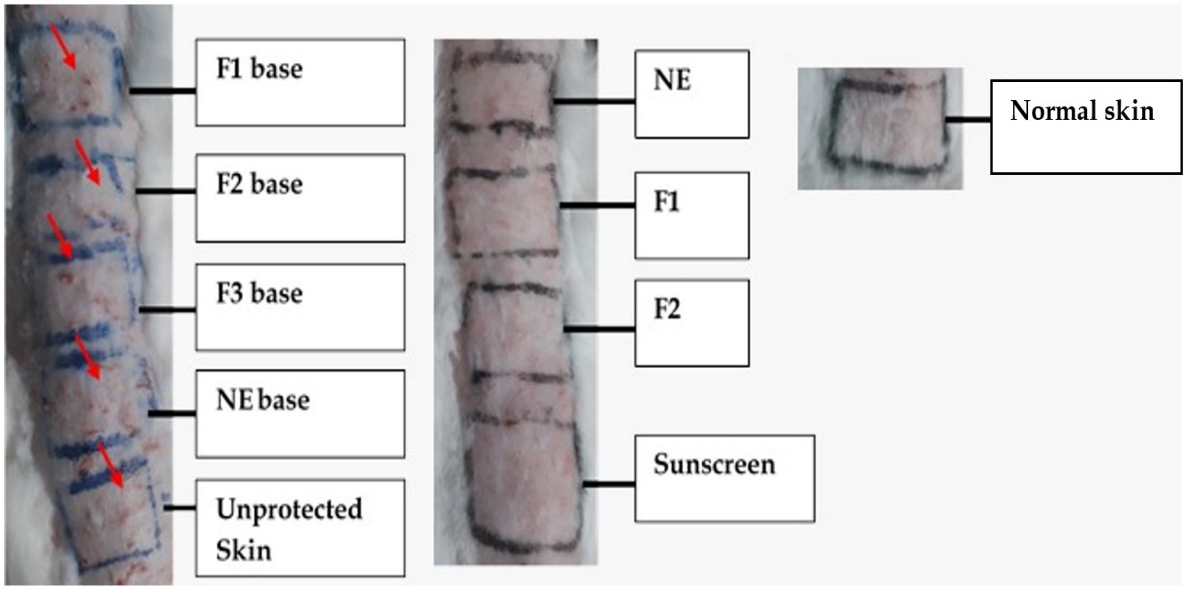

Figure 2. The result of Minimum Erythemal Dose of avocado oil nanoemulsion and nano-emulgel (Red arrow showed the erythema spots)

from nano-emulgel.[42] The release of active substances in the gel is influenced by the polymer matrix of carbopol.[43] Viscosity plays an important role in drug release, where the higher the viscosity of the preparation, the slower the drug is released from the system, which can influence the determination of effectiveness.[44]

Oils containing palmitic acid and linoleic acid have the ability to absorb UV light.[22] The results of in vitro SPF values for nanoemulsion and nano-emulgel formula differ significantly from commercial products. Determining the in vitro SPF value on commercial products differed from the label claims. This is due to the methods used for determining SPF values in industry and laboratories were different.[45] The in vitro test results showed that all formula have an SPF value more than.[14] The samples were categorized as ultra-protection.[46] This not only makes the preparation function as a photo protective agent but also functions to prevent sunburn and UV-induced immunosuppression.[47]

Nanoemulsion and nano-emulgel F1 had the highest MED values compared to F2 and F3 but significantly differed from the positive control. The high MED value indicates that the preparation is quickly absorbed by the skin of the test animals so that it can protect the skin from erythema due to exposure to UV rays. NE and nano-emulgel with 0.5% carbopol can be absorbed more quickly than F2 and F3 which contain the higher carbopol concentrations. The previous research by Hasyim et al. (2019) showed that nanoemulsions can increase the effectiveness of sunscreen due to the rapid release of active substances compared to conventional delivery systems.[48] F2 and F3 had a low MED due to the influence of the viscosity of the thick preparation, so the active substance is difficult to release from the system.[49]

Some studies suggest that additives affect sunscreen effectiveness.[50] This research showed that preparations without avocado oil showed erythema with an average appearance of redness at 13 minutes after exposure to UV light. This indicates that the nanoemulsion and nano-emulgel carriers do not act as sunscreen or inhibit the appearance of erythema, which is in accordance with previous research.[51]

5. Conclusion

Delivery system of avodao oil in nanoemulsion and nano-emulgel showed good physical stability and sunscreen effectivity. Nanoemulsion and nano-emulgel with carbopol at 1 and 1.5% concentrations were stable for 90 days of storage. Avocado oil nanoemulsion has the highest SPF value and categorized as ultra-protection. Carbopol at a 0.5-1.5% concentration can be used as a nano-emulgel base. All these findings indicate that the avocado oil in nanoemulsion and nano-emulgel could be considered for topical application. However, a droplet size and viscosity increase after 90 days of storage was found in carbopol 0.5%.

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