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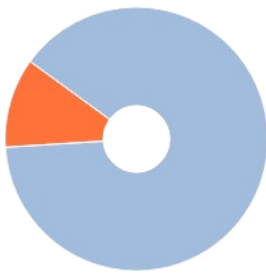
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Stability Study of Avocado Oil Nano-emulgel with Carbopol 980 as A Gel Base

Abstract

Avocado oil (AVO) is a natural source of many unsaturated fatty acids that can help protect the skin from harmful UV radiation. The high content of unsaturated fatty acids in avocado oil can affect product stability. One way to overcome the stability of avocado oil for topical preparation by formulating it into a gel base using Carbopol 980. The purpose of this study was to determine the physical stability and SPF value of avocado oil nano-emulgel (NE) with variations of Carbopol 980 as a gel base for 28 days of storage. AVONE was made with 5% AVO and Carbopol 980 base variation of 0.5% (NE1); 1.0% (NE2;) and 1.5% (NE3.) AVONE was stored in a climatic chamber at $30^{\circ} \pm 2^{\circ}\text{C}$ with RH $65\% \pm 5\%$. The samples were tested for physical and SPF value before storage, day 7, 14, 21, and 28. The data obtained were analyzed statistically using one way Anova. The AVONE on organoleptical parameters were stable with the resulting color is broken-white with a distinctive aroma of oil, thick and homogeneous texture. The pH value ranged from 6.21 ± 0.02 - 7.21 ± 0.02 with all formulas stable during storage. The viscosity value ranged from 13.28 ± 0.23 - 47.22 ± 0.89 dPa.s, the viscosity of NE3 was stable during storage. Adhesion and spreadability showed good ability to adhere to the skin. The SPF values ranged from 8.95 ± 0.43 - 22.41 ± 0.21 and NE3 was stable during storage. Avocado oil nano-emulgel with Carbopol 980 concentration of 1.5% was stable during storage.

Keywords: Avocado Oil; Carbopol; Nano-emulgel

1. Introduction

Avocado oil is a natural source of many unsaturated fatty acids such as oleic, linoleic, and palmitic acids which are ingredients that can help protect the skin from harmful UV radiation (1). Avocado oil is known to contain unsaturated fatty acids with a total content of 85.72%(2). The high content of unsaturated fatty acids in avocado oil causes avocado oil to oxidize very easily so that it can make the oil rancid faster(3). Avocado oil can increase collagen synthesis, reduce the number of inflammatory cells, accelerate the coagulation process, and accelerate epithelial regeneration (4). Avocado oil is a rich source of oleic acid (unsaturated fatty acid) which can increase collagen synthesis, but the content of unsaturated fatty acids in avocado oil can cause oxidation reactions due to its poor stability(5). One way to overcome the stability of avocado oil is to formulate avocado oil into a preparation, namely nano-nano-emulgel (6)(7).

Previous research has formulated avocado oil in several dosage forms such as nanoemulsion (NS), emulgel and nano-emulgel (NE) with Carbopol 940 (7–9). Avocado oil NS showed a good stability and globule size. The avocado oil SPF value and MED (Minimum Erythema Dose) of outcomes are inferior to those of NS, potentially due to the gel base's effect. Consequently, it is essential to investigate the physical stability and SPF value of avocado oil being formulated as a nano-emulgel with carbopol 980 to ensure product stability and efficacy. Nano-emulgels using Carbopol 980 offer advantages over cream formulations, including enhanced stability of the

emulsion system, which is augmented by the increased viscosity of the aqueous phase due to the presence of a gelling agent. This configuration facilitates the delivery of both hydrophilic and hydrophobic compounds, as the nano-emulgel comprises a biphasic system of oil and water (10). Another advantage of the nano-emulgel form is the presence of an oil phase component in the emulsion system, as a good carrier for hydrophobic active substances such as avocado oil, which is difficult if formulated into a form that contains a lot of water such as a gel(11). Nano-emulgel preparations are also known to adhere better than cream preparations, thereby increasing comfort and effectiveness of use (12). A number of different kinds of polymer bases, including Carbopol 980, can be utilized in the production of nano-emulgels.

Carbopol 980 is utilized as a basis owing to its advantages, including a transparent physical appearance of the nano-emulgel, compatibility, stability, and suitable viscosity (13). Carbopol 980 is recognized for providing a formulation that is stable at room temperature and during accelerated storage circumstances (14). Carbopol serves as a base due to its excellent stability, absorption, spreadability, and non-sticky properties, making it very suitable for sunscreen formulations (15). Carbopol 980 used as a gelling agent with a concentration of 0.5% produces an ideal preparation. Carbopol 980 in nano-emulgel preparations produces stable preparations for 3 months at room temperature and accelerated storage conditions (14). Based on the above background, it is necessary to conduct research related to the physical stability and SPF value of avocado oil nanoemulsion in the form of nano-emulgel preparations with variations in Carbopol 980 base.

2. Material and Methods

2.1. Materials

The material used in this study was avocado oil (cosmetic grade) obtained from PT Daarjeling Aroma, Bandung with a certificate of analysis. The carrier materials were Tween 80, Span 80, Sorbitol, Paraffin Liquid, TEA, methyl paraben, propyl paraben, Carbopol 980 obtained from PT Multi Kimia Raya Semarang with cosmetic grade. DMSO and distilled water were obtained from PT Multi Kimia Raya Semarang with analytical grade.

The tools used in this study are a set of climatic chamber (Taisite HWS-70BX®), viscometer (Rheosys Merlin II®) with type of cone and plate, a set of adhesion test equipment, a set of spreadability test equipment, and UV-Vis spectrophotometer (Shimadzu UV-1800®).

2.2. Methods

The preparations of nano-emulgel was begun with nanoemulsion production (Table 1). Tween 80 and PEG 400 were heated at 35°C in a different beaker glass. Tween 80 and PEG 400 were mixed using magnetic stirrer at 700 rpm. The avocado oil then added drop wise into the

mixture and stirred at 1200 rpm until homogenous and showed a clear and transparent nanoemulsion. The product was tested for particle size, polydispersity index and zeta potential to ensure that the preparation falls into nanoemulsion category.

Table 1. Formula of avocado oil nanoemulsion

Materials	Concentration (%b/v)
Avocado oil	7.5
Tween 80	30
PEG 400	40
Distilled water	Up to 100

The preparation of nano-emulgel begins with weighing each ingredient according to the formula on table 2. The gel phase (Carbopol 980) was made by dispersing the polymer base (Carbopol 980) into hot water at 80°C and stirring in a mortar until a thick gel mass was formed. The emulsion phase was then made consisting of an aqueous phase (methyl paraben, and tween 80) and an oil phase (propyl paraben, liquid paraffin, and span 80), each phase of which was first homogenized in a waterbath that had been adjusted to a temperature of 70°C. The oil phase was added to the water phase in a glass beaker. The mixture was stirred using a magnetic stirrer that had been set at 40°C and a speed of 500 rpm, then distilled water and sorbitol were added, and stirred until homogeneous, after which avocado oil was added little by little with stirring until homogeneous. The homogeneous emulsion phase is mixed with the gel base (Carbopol 980) that has been formed. Each formula was produced for 3 replications.

Table 2. Formula of avocado oil nano-nano-emulgel with Carbopol 980 as a gel base

Materials	Concentration (%b/v)		
	NE1	NE2	NE3
Avocado oil nanoemulsion	5	5	5
Carbopol 980	0,50	1,00	1,50
Tween 80	17,50	17,50	17,50
Span 80	2,50	2,50	2,50
Sorbitol	1,00	1,00	1,00
Paraffin Liquid	1,25	1,25	1,25
Methyl parabene	0,18	0,18	0,18
Propyl paraben	0,02	0,02	0,02

Triethanolamine	0,80	0,80	0,80
Distilled water		Up to 100	

Avocado oil nano-emulgel was stored in a climatic chamber at $30^{\circ} \pm 2^{\circ}\text{C}$ with RH 65% \pm 5%. Samples were tested before storage and on days 7, 14, 21, 28 (13)(16). The parameters tested were:

2.2.1 Organoleptical Test

Organoleptical testing is done by direct observation using the five senses including color, odor and consistency of the nano-emulgel made (17).

2.2.2. Homogeneity Test

An nano-emulgel preparation of 0.1 g was applied to a transparent glass plate and observed for homogeneity. The test preparation must show a homogeneous arrangement, indicated by the absence of coarse grains on the glass object (18).

2.2.3. pH test

The pH measurement was carried out using a pH meter, at room temperature. Before use, the pH meter electrode is washed and rinsed with distilled water and then dried. The tool is calibrated using a standard buffer solution of pH 4 and pH 7 (BPOM RI, 1995). The pH test is also carried out to determine if the pH of the nano-emulgel preparation meets the requirements according to SNI 16-3499-1996 a good pH for the skin is 4.5-8 (19).

2.2.4. Viscosity test

The viscosity test was carried out with a Rheosys Merlin II type cone and plate viscometer. A sample of 0.5 g was put into the plate and then the cone was positioned to start the measurement. Viscosity and flow curves are generated automatically with the Rheosys Micra application (13).

2.2.5. Adhesion Test

The adhesion test is carried out by weighing the preparation as much as 0.25 grams placed on a glass object that has been determined in area then another glass object is placed above. The glass object was then mounted on the test device and given a load of 1 kg for 5 minutes and then released with a load weighing 80 grams. The time is recorded until the two glass objects are released (20).

2.2.6. Spreadability Test

A total of 0.5 grams of nano-emulgel preparation was placed in the center of a transparent glass covered with graph paper underneath, then covered with another transparent glass on top, given a load (50 g, 100 g, 150 g, 200g and 250g) and allowed to stand for 1 minute, measuring the diameter of the nano-emulgel spread area released (18).

2.2.7. SPF Value Test

Determination of SPF value was done using UV-Vis spectrophotometer by weighing 1 gram of avocado oil nano-emulgel then put into a 10 ml volumetric flask and dissolved with DMSO as much as 5 ml. The solution was vortexed until completely dissolved. The solution that has been obtained is measured with a UV-Vis spectrophotometer at a wavelength of 290-320 nm using DMSO as a blank, then the absorbance value is recorded at every 5 nm interval. The absorbance value was calculated using the Mansur equation(21) as follows:

$$SPF = CF \times \Sigma 290320 EE(\lambda) \times I(\lambda) \times Abs(\lambda) \quad (1)$$

Note:

CF = Correction factor

Abs (λ) = Absorption of sunscreen product

$\Sigma 290320 EE(\lambda)$ = Erythema effect spectrum

I (λ) = Intensity of light spectrum

2.3. Data Analysis

Organoleptic and homogeneity test data were described descriptively, while pH, viscosity, adhesion, spreadability, and SPF values were analyzed statistically using one way Anova to detect any physical changes in all formulas during 28 days of storage. Nano-emulgel is concluded as a stable product if there is no significant changes or significance value were > 0.05.

3. Results and Discussion

The result of the nanoemulsion of avocado oil can be seen in Figure 1 and Table 3. The product was categorized as nanoemulsion according to its particle size.

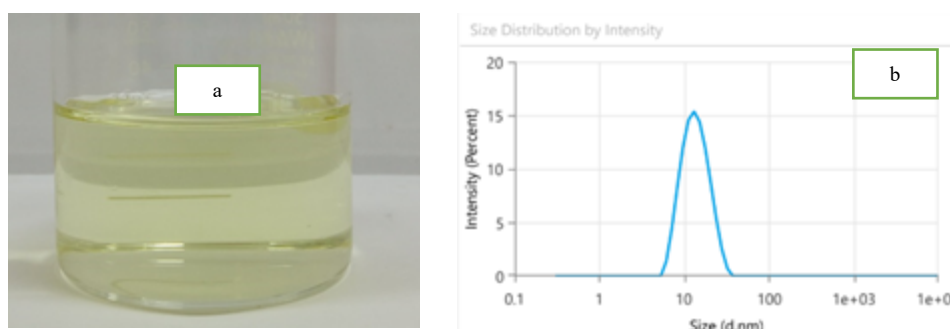


Figure 1. The result of organoleptic test (a) and particle size (b) of avocado oil nanoemulsion

Table 3. The result of physical test of avocado oil nanoemulsion

Parameters	Result
Physical appearance	Clear and transparent liquid

Particle size (nm)	12.74 ± 0.24
Polydispersity index	0.1415 ± 0.02
Zeta potential (mV)	22.5 ± 1.15

Based on the result above, it can be concluded that the preparation was categorized as a nanoemulsion. The result of the particle size was below 100 nm and polydispersity index was below 0.5 showing that the nanoemulsion had a homogenous globule size (22). The zeta potential was below 30 mV indicating that the nanoemulsion had a good stability(23). Tween 80 with PEG 400 can create transparent and stable nanoemulsions close to a micellar system(24). Tween 80 is a hydrophilic surfactant that effectively decreases surface tension in nanoemulsion formulations (25).

The results of the organoleptical test of avocado oil nano-emulgel preparations for NE1, NE2 and NE3 can be seen in Figure 2.



Figure 2. The result of the organoleptic test of avocado oil nano-emulgel with Carbopol 980 (a: before storage; b: after the storage)

The results of organoleptical testing of avocado oil NE did not show any color changes during 28 days of storage. The texture of the preparation in NE1 was slightly thick while NE2 and NE3 had a thicker texture. The difference in texture of the three formulas was due to the different concentration of Carbopol 980 base used in each formula. According to research by Nabillah et al. (2022), the greater the concentration of gelling agent used, the thicker the texture of the preparation will be. The three formulas after 28 days of storage became thinner in texture. This is because gel preparations might experience a syneresis. This event was caused by the release of water from the preparation where the preparation shrinks so that it tends to squeeze water out of the preparation (Kuncari et al., 2014).

The homogeneity test results of avocado oil nano-emulgel preparations for NE1, NE2 and NE3 can be seen in Figure 3.

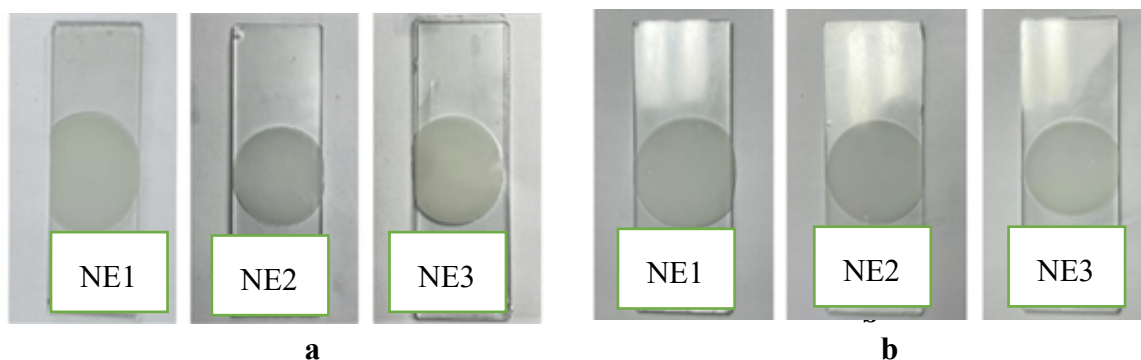


Figure 3. The result of Avocado oil nano-emulgel homogeneity test results before storage (a) and after the storage (b)

NE1, NE2 and NE3 avocado oil nano-emulgel obtained homogeneous results for 28 days of storage as shown in the picture above. All formula showed the absence of coarse grains or unmixed particles. The preparation exhibited inhomogeneity, as evidenced by the presence of coarse grains on the glass object (18). The results of the homogeneity test obtained are in line with the research conducted by Nabillah et al (2022) showing that the different concentrations of Carbopol 980 have no effect on the homogeneity of the preparation.

The pH test results of avocado oil nano-emulgel preparations for NE1, NE2 and NE3 can be seen in table 4.

1

Table 4. The result of pH stability of avocado oil nano-emulgel with Carbopol 980 as a gel base

Day	pH		
	NE1	NE2	NE3
Before Storage	7.21±0.02	6.87±0.01	6.21±0.02
7	7.20±0.02	6.89±0.01	6.24±0.02
14	7.21±0.02	6.87±0.01	6.24±0.04
21	7.20±0.02	6.86±0.03	6.23±0.02
28	7.21±0.02	6.86±0.03	6.22±0.01
p value	0.543	0.671	0.774

Data displayed n = 3 ±SD

The pH test results obtained show that NE1 has a higher pH than NE2 and NE3. This indicates that increasing the concentration of Carbopol 980 can affect the pH of the nano-emulgel preparation. The greater the concentration of Carbopol 980 used, the lower the pH will be. avocado oil nano-emulgel pH in NE1, NE2 and NE3 obtained results between 6.21 ± 0.02 - 7.21 ± 0.02 . The pH value obtained is in accordance with the range of pH requirements (SNI) for topical preparations, namely 4.5-8.

Based on the statistical analysis, the results did not show any significance difference in pH ($p>0.05$) in the three formulas, which means that there is no significant difference in the pH value of avocado oil nano-emulgel preparations during the storage cycle, so it can be said that all formula are stable for 28 days of storage.

The viscosity test results of avocado oil nano-emulgel preparations for NE1, NE2 and NE3 can be seen in table 2.

1

Table 5. The result of viscosity test of avocado oil nano-emulgel with carbopol 980 as a gel base

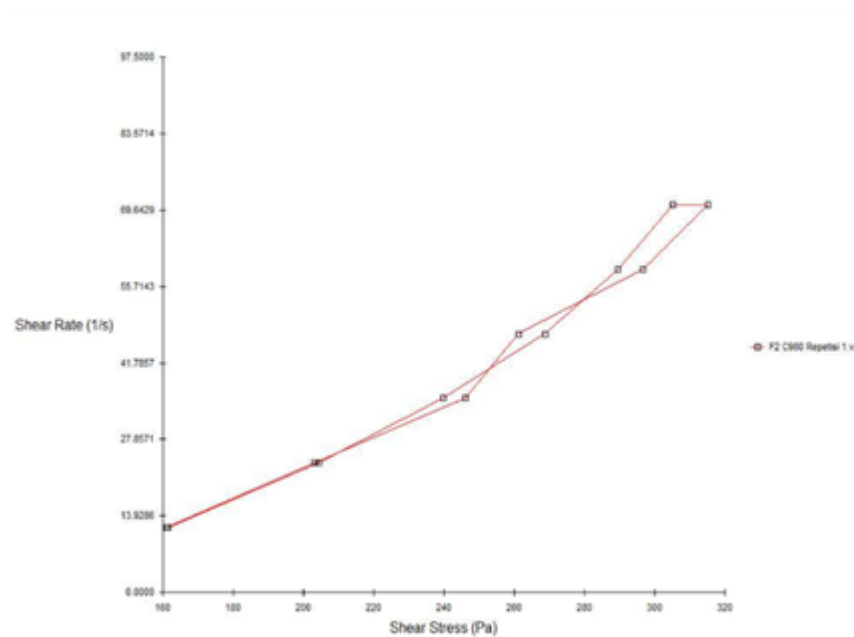
Day	Viscosity (dPa.s)		
	NE1	NE2	NE3
Before Storage	16.88±0.08	43.16±0.07	45.33±0.66
7	15.61±0.22	40.65±0.25	46.42±0.91
14	15.48±0.15	39.32±0.22	46.38±0.82
21	14.31±0.25	38.35±0.41	47.30±0.78
28	13.28±0.23	37.45±0.28	47.22±0.89
p value	0.001	0.024	0.512

Data displayed n = 3 ±SD

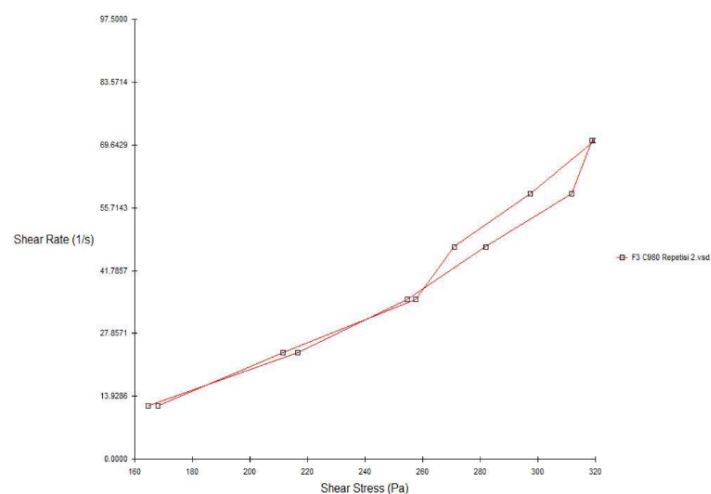
The viscosity results showed changes during 28 days of storage in NE1, NE2 and NE3, but the changes in the three formulas are still within the range that meets the requirements for good nano-emulgel viscosity values, namely 3-300 dPa.s (27). Based on the results of the viscosity values obtained, it showed that NE3 has a higher viscosity value than NE1 and NE2 and this is in line with previous research conducted by Nabillah et al (2022) , which shows that the higher the concentration of Carbopol 980, the viscosity value of the preparation obtained increases. The increasing viscosity value indicates that the thicker a preparation is (28).

Based on the statistical analysis, the viscosity value of NE3 did not showed any significance difference ($p>0.05$) which means that there was no significant difference in the viscosity value of avocado oil nano-emulgel preparations during storage. In NE1 and NE2, there were a significance changes ($p<0.05$) in the viscosity values of avocado oil nano-emulgel preparations during storage. Both NE1 and NE2 experienced a decrease in viscosity during storage. According to research by Rismawati et al., (2020), this decrease in viscosity can occur due to the longer temperature and storage time. This is because the nano-emulgel experiences syneresis. Syneresis occurs due to the matrix structure or gel fibers that persist in solidifying, ultimately leading to the expulsion of water and enabling the liquid to migrate to the surface(30,31). This might be caused by the container system(31).

(a)

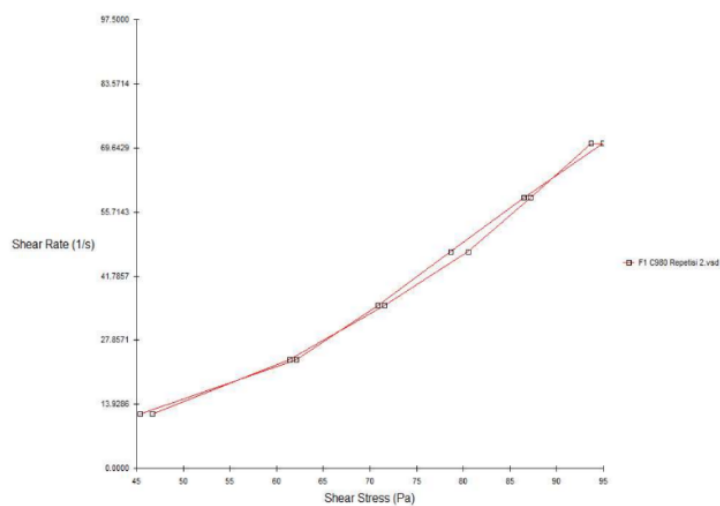


(b)



(c)

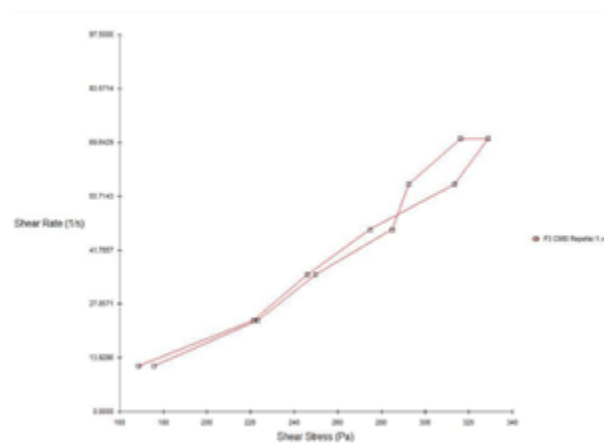
Figure 4. Rheogram of avocado oil nano-emulgel with carbopol 980 as a gel base before storage (a=NE1; b=NE2; c=NE3)



(a)



(b)



(c)

Figure 5. Rheogram of avocado oil nano-emulgel with carbopol 980 as a gel base after storage (a=NE1; b=NE2; c=NE3)

The rheogram results show that the rheology of nano-emulgel NE1, NE2 and NE3 were categorized as pseudoplastic. Pseudoplastic are characterized by a slightly curved upward graph shape (32). Pseudoplastic flow is characterized by a flow curve that intersects the origin (0,0), in contrast to plastic flow, indicating that pseudoplastic flow lacks a yield value. The viscosity of pseudoplastic substances decreases with increasing rate of shear. This occurs in long-chain molecules such as polymers. The pseudoplastic system is also called a dilute shear system because by increasing the shear stress the viscosity decreases (26). The rheology profile of all formula did not show any changes. The rheology of Carbopol 980 is not affected by temperature and storage duration but is affected by the cross-linker used during formulation (33).

The results of the avocado oil nano-emulgel preparation adhesion test for NE1, NE2 and NE3 can be seen in table 6.

Table 6. The result of adhesion test of avocado oil nano-emulgel with carbopol 980 as a gel base

Day	Adhesion (minute)
-----	-------------------

	NE1	NE2	NE3
Before Storage	5.43±0.07	15.20±0.08	30.34±0.07
7	5.40±0.11	15.32±0.14	30.27±0.14
14	5.81±0.09	15.34±0.09	30.25±0.08
21	4.76±0.03	14.74±0.06	30.27±0.15
28	4.22±0.18	14.36±0.26	30.31±0.20
p value	0.000	0.000	0.541

Data displayed n = 3 ±SD

The results of the avocado oil nano-emulgel adhesion test showed that NE3 had a longer adhesion time than NE1 and NE2, but the adhesion of all formulas still met the requirements for good topical preparation adhesion, which is not less than 4 seconds. The results of the avocado oil nano-emulgel adhesion test obtained show that the higher the concentration of Carbopol 980, the more the viscosity of the resulting preparation will increase so that the adhesion time will be longer (34).

Based on the results of statistical analysis, the adhesion value of avocado oil nano-emulgel preparations shows significance results ($p>0.05$) in NE3, which means that there is no significant difference in the adhesion value of avocado oil nano-emulgel preparations during the storage cycle, so it can be said that NE3 is stable for 28 days of storage. In NE1 and NE2, the significance results ($p<0.05$) mean that there are significant differences in the adhesion value of avocado oil nano-emulgel preparations during the storage cycle, so it can be said that NE1 and NE2 are not stable during 28 days of storage. The adhesion value started changing in day 21. The adhesion values of NE1 and NE2 have decreased during storage due to a reduction in viscosity. However, the adhesion values remain adequate for effective topical preparation, not falling below 4 seconds (34).

The results of the spreadability test of avocado oil nano-emulgel preparations for NE1, NE2 and NE3 can be seen in table 4.

Table 7. The result of spreadability test of avocado oil nano-emulgel with Carbopol 980 as a gel base

Day	Spreadability (cm)		
	NE1	NE2	NE3
Before Storage	5,14±0,10	3,72±0,02	3,54±0,02
7	5,19±0,10	3,75±0,04	3,57±0,05
14	5,17±0,13	3,85±0,02	3,57±0,05
21	5,45±0,02	4,00±0,02	3,53±0,02
28	5,38±0,02	3,97±0,01	3,61±0,03
p value	0.025	0.012	0.146

Data displayed n = 3 ±SD

The spreadability of the skin preparation is crucial to evaluate, as it pertains to the user's ease of application. The broader the active material, the more effectively it will be dispersed (34). The results of the avocado oil nano-emulgel spreadability test showed that NE1 has the ability to spread more widely than NE2 and NE3. The results showed that the higher the concentration of gelling agent, the smaller the spreadability value obtained. The results are due to the interconnection between spreadability and viscosity. As the viscosity of the preparation increases, its spreadability decreases; conversely, a decrease in viscosity results in enhanced spreadability (29).

Based on the results of statistical analysis, the value of the spreadability of avocado oil nano-emulgel shows significance results ($p > 0.05$) in NE3, which means that there was no significant difference in the value of the spreadability of avocado oil nano-emulgel preparations during the storage cycle, so it can be said that NE3 is stable for 28 days of storage. Whereas in NE1 and NE2 the significance results ($p < 0.05$) mean that there were a significant difference in the value of the spreadability of avocado oil nano-emulgel preparations during the storage cycle. The spreadability values of NE1 and NE2 increased during storage, influenced by viscosity and reduced adhesion. However, spreadability should not be considered absolute data, as there is no literature providing an exact ideal value (34). The results of the SPF value test for avocado oil nano-emulgel preparations for NE1, NE2 and NE3 can be seen in table 8.

Table 8. The result of SPF value of avocado oil nano-emulgel with carbopol 980 as a gel base

Day	SPF Value		
	NE1	NE2	NE3
Before Storage	9,02±0,81	11,93±0,38	22,38±0,24
7	8,95±0,43	12,01±0,36	22,41±0,21
14	9,65±0,09	11,96±0,08	22,34±0,32
21	9,71±0,27	12,01±0,23	22,42±0,27
28	9,80±0,18	11,98±0,02	22,37±0,23
p value	0.468	0.255	0.107

The results of the SPF value showed that all of the formula were categorized as the results maximum protection. This is in line with previous research conducted by Shabrina et al (2024) on nanoemulsion and nanoemulgel preparations using 5% avocado oil which resulted in the maximum level of protection category(8). The SPF values measured over a 28-day storage period in NE1 and NE2 exhibited fluctuations, while NE3 maintained stable. There are several things that factor into changes in the SPF value of a preparation including the type of sunscreen

solvent, concentration and combination of sunscreens, type of emulsion, and the interaction and effect of other formulation components including esters, emollients, and emulsifiers which are some of the elements that affect the estimated SPF value. Increased or decreased absorption of UV radiation may be due to some of these variables(36).

Based on the results of statistical analysis, the SPF value of avocado oil nano-emulgel preparations showed significance results ($p>0.05$) in all formula, which means that there is no significant difference in the SPF value of avocado oil nano-emulgel preparations during the storage cycle.

Nano-emulgel with Carbopol 980 base obtained physically stable results. The results obtained by nano-emulgel are in accordance with the previously determined TPP (target product profile) (37). Carbopol 980 was found to be the best in modifying the rheological properties and viscosity of nanoscale emulsions designed for topical applications(38). Carbopol 980 demonstrated favorable test outcomes, indicating its potential as an effective carrier for topical formulations (39). This research is in line with the previous result that using 1.5% w/v Carbopol 980 as a gelling agent resulted in rheologically stable results and was acceptable for topical application (40,41). Increasing the concentration of Carbopol 980 as a gelling agent can improve the physical properties of the preparation(42). Carbopol 980 as a gelling agent with a concentration of 1.5% has good stability and does not show significant physical/chemical changes in nano-emulgel preparations (43).

4. Conclusion

Based on the result above, it can be concluded that avocado oil in nano-emulgel preparation with 1,5% of Carbopol 980 was stable during 28 days of storage at the temperature of $30\pm 2^{\circ}\text{C}$ and RH of $65\pm 5\%$.

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Conflict of Interest

All authors declared that there was no conflict of interest.

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