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Preparation and Evaluation of Nanoemulgels Containing a Combination of Grape Seed Oil and Anisotriazine as Sunscreen

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Abstract

BACKGROUND: Grape seed oil contains Vitamin E which acts as skin antioxidant and natural ultraviolet (UV) absorbent and anisotriazine is used as chemical absorbent. Sun protection factor (SPF) value of the sunscreen and physical stability can be increased using a combination of grape seed oil and anisotriazine as sunscreen material and preparation by nanotechnology.

AIM: The objective of this study was to prepare and evaluate physical stability and *in vitro* SPF value of sunscreen nanoemulgel containing grape seed oil and anisotriazine.

METHODS: Nanoemulgels containing 4% grape seed oil and anisotriazine (1.6% and 3.2%) were formulated by adding 2% of Carbopol 940 gel to the optimized nanoemulsions formulation with a ratio of nanoemulsion and gel 4:1. The nanoemulgels were evaluated physical stability during storage for 12 weeks at variations of temperature, centrifugation, and cycling test. SPF values of nanoemulgels were determined by UV–visible spectrophotometric method and compared to emulgel. Droplet morphology observation of nanoemulgel using transmission electron microscope.

RESULTS: The results of this study showed that sunscreen nanoemulgel containing 4% grape seed oil and 3.2% anisotriazine had average droplet size of 187.5 nm, physically stable during experiment for 12 weeks at variation of temperature and after centrifugation and cycling test, but the sunscreen emulgel showed a phase separation. The SPF of nanoemulgel containing a combination of 4% grape seed oil and 3.2%, nanoemulgel without anisotriazine, and emulgel formulation was 19.325 ± 0.232, 11.169 ± 0.113, and 11.913 ± 0.161, respectively. Transmission electron microscopy analysis of droplet morphology showed that this nanoemulgel formulation formed a spherical globule.

CONCLUSION: The sunscreen nanoemulgel formulation containing combination of 4% grape seed oil and 3.2% anisotriazine more stable than sunscreen emulgel during experiment for 12 weeks at room temperature and showed the SPF value higher compared to emulgel containing 4% grape seed oil and 3.2% anisotriazine and nanoemulgel without anisotriazine.

Introduction

The ultraviolet (UV) radiation (UVR) caused sunburn damage, photoaging, and skin cancer [1], [2], [3]. UVR can produce free radical oxygen species and damage the DNA of the skin. The deleterious effects of UVR are inhibited through the use of sunscreens [4]. Some chemical UV filters showed the adverse effects of sunscreen product such as irritant, allergic, phototoxic, and photoallergic [5]. Therefore, this research used grape seed oil to increase safety of sunscreen product. The use of grape seed oil (vegetable oil) could be a promising strategy to improve the SPF of sunscreen formulation with a reduction in content of anisotriazine. The sunscreen with high sun protection factor (SPF) can be obtained with nanotechnology formulations and combinations of sunscreen [6].

In this study, grape seed oil is used as natural UVB absorbent and combined with anisotriazine as a chemical UVA and UVB absorbent. Anisotriazine is an oil-soluble material and photostable. The solubility of the lipophilic ingredient of anisotriazine is improved by nanoemulsion formulation [7], [8].

Grape seed oil is a yellowish oil and odorless and rich in antioxidant compounds such as tocopherols (Vitamin E), tocotrienols, flavonoids, phenolic acids, and carotenoids so it is very well used in cosmetic formulations [9]. Antioxidants can provide a protective effect on the skin against damaging effect UVR mediated free radicals [10]. Oxidation of cellular biomolecules caused by acute exposure of human skin could be prevented by antioxidant, acting as free radical scavenger [3]. Vitamin E also absorbs strongly in the UVB region at wavelengths of 280–320 nm [11].

The SPF value of the sunscreen and physical stability can be increased using nanotechnology. Nanoemulsion has droplet size of 20–500 nm smaller than conventional emulsion that had droplet size of 0.1–100 μm, so nanoemulsion more stable than emulsion. Nanoemulgel known as hydrogel-based nanoemulsion, with the presence of a thickening agent, the stability of the nanoemulgel preparation will be better due to a decrease in interface tension and an increase in viscosity and adhesion at the time of topical administration. Furthermore, nanoemulgel shows the benefits of being non-greasy, easily spread, and clean [12]. Thus, the aim of this research was to formulate and evaluate physical stability and determined SPF value of sunscreen nanoemulgels containing a combination of grape seed oil and anisotriazine by a simple and reliable UV–visible spectrophotometry.

Materials and Methods

The materials used in this study were grape seed oil (Aceites Borges Pont, S.A.U., Spanyol), anisotriazine (Ashland, America), Tween 80, and sorbitol which were purchased from PT. Bratachem, Medan, methylparaben, propylparaben, Span 80, propylene glycol, CMC Na, glycerol, ethanol, Carbopol 940, and triethanolamine (TEA) were purchased from CV. Rudang Jaya, Medan. These materials were analytical grade.

The nanoemulsions containing grape seed oil were prepared with surfactants (Tween 80) and cosurfactants (sorbitol) using the high-energy emulsification method [13], [14]. In this study, the preparation of nanoemulsions using magnetic stirrer and ultrasonicator. The aqueous phase was prepared by dissolving preservative (methylparaben and propylparaben) in water and Tween 80 was added to this solution. The mixture of grape seed oil and sorbitol was mixed to the water phase and stirred at 2400 rpm for 6 h by 79-1 laboratory magnetic stirrer with heater (China). Then, the nanoemulsions were sonicated for 1 h by Ultrasonic Cleaner Branson 1510 E-MT (USA) until transparent nanoemulsions were produced. The optimum nanoemulsion formulation was selected based on the smallest droplet size used for the preparation of nanoemulgel containing 5% grape seed oil and anisotriazine 2 and 4%.

The 2% Carbopol 940 solution was used to prepared the nanoemulgels containing grape seed oil and anisotriazine. First, Carbopol 940 was dispersed in pure water and added with TEA to pH 6–6.5 to obtain the gel base of 2% Carbopol 940 solution. Nanoemulgels were prepared by mixing the obtained nanoemulsions containing grape see oil and anisotriazine with the gel base of 2% Carbopol 940 (ratio of nanoemulsion and gel 4:1), then stirred using a magnetic stirrer with heater at 2400 rpm for 8 hours and ultrasonicated until a transparent nanoemulgels were produced.

The preparation of emulsion containing 4% grape seed oil and 3.2% anisotriazine was prepared by dissolving anisotriazine and Span 80 in grape seed oil and then heated to 70°C. The preservatives (methyl and propyl parabens) were dissolved in propylene glycol and glycerol and added CMC Na solution and this mixture was heated to 70°C. After that, the mixture of anisotriazine, Span 80, and grape seed oil was added to the mixture of preservative and CMC Na solution and stir with magnetic stirrer for 45 min [15]. Emulgel was obtained by mixing the obtained emulsions with a gel base of 2% of Carbopol 940 (ratio of emulsion and gel 4:1) with gentle stirring for 10 min [16].

The mean droplet size for nanoemulgel formulations was measured by laser light scattering using the Nanoparticle Analyzer HORIBA SZ-100, Germany. The pH measurement is done using 1% nanoemulgel solution in pure water with a digital pH meter (Hanna instrument).Viscosity measurement was carried out using the Brookfield DF-E Viscometer and the sunscreen nanoemulgel was measured every 2 weeks for 12 weeks of storage at room temperature.

Physical stability evaluation of nanoemulgels was done by storing its at 28 ± 2°C (room temperature), 40 ± 2°C (high temperature), and 4 ± 2°C (low temperature) for 12 weeks. Nanoemulgel formulas were observed through visual inspection for their color, odor, and phase separation with observation every week [16], [17].

Cycling test for sunscreen nanoemulgels and emulgel was done by putting them in the freezer at 4 ± 2°C for 24 h and then put in Climatic Chamber Memmert, Germany, at 40 ± 2°C for 24 h and repeated in 6 cycles. After that, the physical stability was observed. Centrifugation test was done using centrifuges (Hitachi CF 16 R X II, Japan) with a rotation of 3750 rpm during for 5 h at 25 ± 2°C. The physical stability of the nanoemulgel was observed [17].

The morphology and globule size of nanoemulgel containing 4% grape seed oil and 3.2% anisotriazine were analyzed using transmission electron microscope (JEOL JEM 1400, Japan).

The sunscreen nanoemulgel and emulgel for SPF determination were diluted to volume with 96% ethanol to the final concentration of the sample which was 200 μg per mL. The absorption spectra were obtained in the range of 290–320 nm with an interval 5 nm measurement using a spectrophotometer UV–visible (Shimadzu UV 1800, Japan) and six determinations were made for each sample [18].

The SPF value was calculated using the following equation:

$$SPF = CF \times \sum_{290}^{320} \times EE(\lambda) \times I(\lambda) \times Abs(\lambda)$$

Where: CF: Correction factor (=10); EE: Erythemal effect spectrum; I: Solar intensity spectrum;

Abs: Absorbance of sample. The absorbance value obtained is multiplied by EE x I for each interval [19].

Results

The nanoemulsions were prepared in three formulations using a ratio variation of Tween 80 as surfactant and sorbitol as cosurfactant is presented in Table 1.

Table 1: Composition of nanoemulsions containing grape seed oil

Ingredients	Quantity of 100 mL (%)		
	F1	F2	F3
Grape seed oil	5	5	5
Tween 80	25	26	27
Sorbitol	35	34	33
Methylparaben	0.1	0.1	0.1
Propylparaben	0.02	0.02	0.02
Distilled water to	100	100	100

The sunscreen nanoemulsion formulations (F1, F2, and F3) showed a light yellow color and transparency, as shown in Figure 1. Formulation of F3 that used the highest Tween 80 (surfactant) shows the lowest of droplet size (9.2 nm) and this formula was selected for preparation nanoemulsion and nanoemulgel containing combination of grape seed oil and anisotriazine.

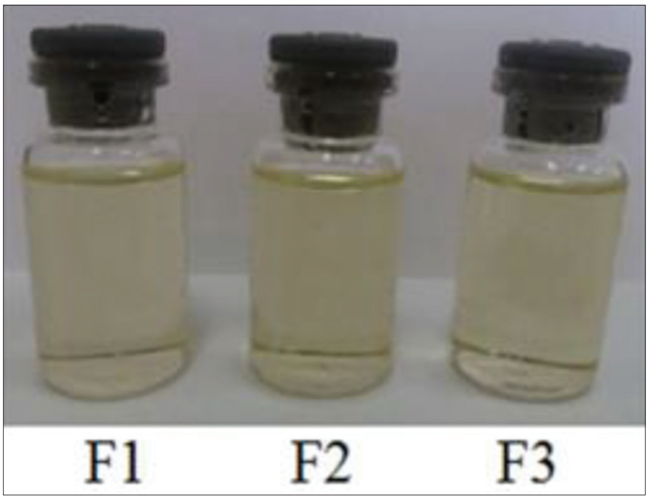

Figure 1: Formulation of nanoemulsion containing 5% grape seed oil with a ratio variation of Tween 80 and sorbitol F1 (25:35), F2 (26:34), F3 (27:33)

The composition of nanoemulgels containing grape seed oil and anisotriazine is presented in Table 2. All sunscreen nanoemulgel formulations (F4, F5, and F6) had a transparent appearance, slightly yellow in color and stable during storage for 12 weeks under varied temperature condition, as shown in Figure 2.

The composition of emulgel containing grape seed oil and anisotriazine is presented in Table 3. The result of emulgel formulation was milky white. The droplet size of this emulgel formulation was larger than nanoemulgel, therefore, the appearance of emulgel was not transparent. The physical stability evaluation of nanoemulgel containing grape seed oil and anisotriazine showed that the nanoemulgel was stable, there was no phase separation, while the emulgel formulation showed the phase separation after storage for 7 weeks at room temperature as it is shown in Figure 3. It can be explained that nanoemulsions prepared using a magnetic stirrer at 2400 rpm and ultrasonicator was found to be more stable for longer duration of time when compared to emulsions prepared by mechanical agitation [20].

Table 2: Composition of grape seed oil and anisotriazine nanoemulgels

Ingredients (% wt/wt)	Quantity of 100 mL (%)		
(Nanoemusion)	F4	F5	F6
Grape seed oil	5	5	5
Anisotriazine	0	2	4
Tween 80	27	27	27
Sorbitol	33	33	33
Methylparaben	0.10	0.10	0.10
Propylparaben	0.02	0.02	0.02
Distilled water to	100	100	100
Ingredient (% wt/wt) (Gel base)			
Carbopol 940	2		
TEA	1		
Distilled water to	100 mL		

TEA: Triethanolamine.

Table 3: Composition of grape seed oil and anisotriazine emulgel

Ingredients (emulsion)	Quantity of 100 mL (%)
Grape seed oil	5
Anisotriazine	4
Tween 80	1.25
Span 80	3.73
Methylparaben	0.10
Propylparaben	0.02
Propylene glycol	8
Glycerin	10.4
CMC Na	0.8
Distilled water to	100
Ingredients gel base	
Carbopol 940	2
TEA	1
Distilled water to	100 mL

TEA: Triethanolamine.

The pH of the nanoemulgel and emulgel containing combination of grape seed oil and anisotriazine decreased during the storage of 12 weeks as it is shown in Table 4, but the pH values were within the range of pH skin, normal from 4.5 to 6.0, which are considerably acceptable and less irritable for use in human skin [21].

Table 4: pH of nanoemulgel and emulgel at storage for 12 weeks at room temperature

Week	Average pH			
	F4	F5	F6	Emulgel
0	7.03 ± 0.00	7.06 ± 0.11	7.20 ± 0.10	7.03 ± 0.06
2	7.00 ± 0.00	7.03 ± 0.06	7.00 ± 0.00	6.96 ± 0.06
4	6.96 ± 0.06	6.93 ± 0.06	6.93 ± 0.06	6.86 ± 0.06
6	6.86 ± 0.06	6.83 ± 0.06	6.83 ± 0.06	6.73 ± 0.06
8	6.66 ± 0.06	6.60 ± 0.10	6.56 ± 0.11	6.43 ± 0.05
10	6.46 ± 0.06	6.40 ± 0.10	6.36 ± 0.06	6.10 ± 0.10
12	6.23 ± 0.06	6.20 ± 0.10	6.13 ± 0,06	5.76 ± 0.06

The droplet size of all nanoemulgels <200 nm was increased after storage for 12 weeks at room temperature, but the size of droplets was still smaller than 500 nm, as shown in Figure 4. The nanoemulsion is obtained when the size of droplet reaches approximately 20–500 nm [22]. Otherwise, the droplet size of the emulgel was larger than nanoemulgel.

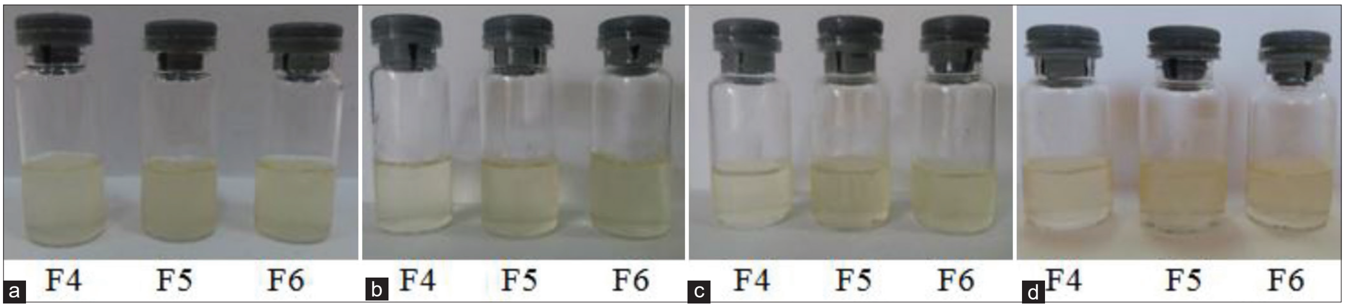

Figure 2: Formulation of sunscreen nanoemulgel (F4: Containing 4% grape seed oil only, F5: Containing 4% grape seed oil and 1.6% anisotriazine, F6: Containing 4% grape seed oil and 3.2% anisotriazine), (a) before storage, (b) after storage for 12 weeks at room temperature, (c) after storage for 12 weeks at high temperature, (d) after storage for 12 weeks at low temperature

Figure 3: Formulation of sunscreen emulgel containing 4% grape seed oil and 3.2% anisotriazine (a: Before storage, b: After storage for 7 weeks at room temperature)

Therefore, the emulgel was not physically stable during 12 weeks storage at room temperature.

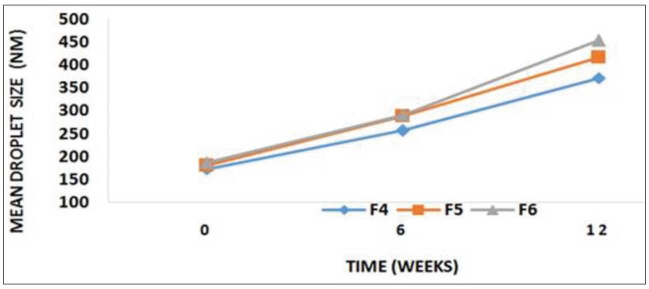

Figure 4: Droplet size of sunscreen nanaoemulgels (F4, F5, and F6)

All nanoemulgel preparations (F4, F5, and F6) remained stable after centrifugation test with no sedimentation and creaming or phase separation observed in Figure 5, but the emulsion was not stable with the formation of phase separation as it is shown in Figure 6. The centrifugation test was performed to determine the effect of gravity on the stability of the nanoemulgel which is equivalent to the gravitational force for a year.

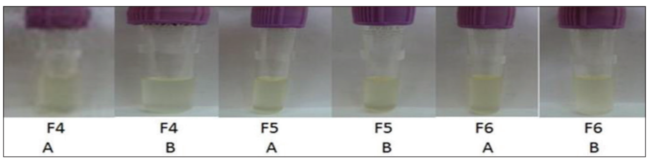

Figure 5: Appearance of sunscreen nanoemugel formulations after centrifugation

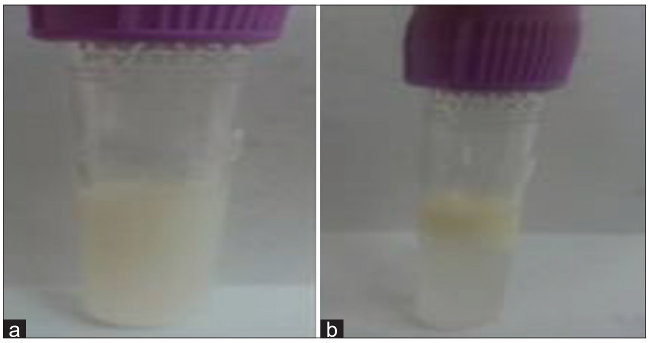

Figure 6: Appearance of emulgel formulation containing 4% grape seed oil and 3.2% anisotriazine after centrifugation test (a: Before centrifugation, b: After centrifugation)

The viscosity of the nanoemulgel formulations (F4, F5, and F6) showed an increase in viscosity value for 12 weeks of storage, while the emulgel showed a decrease in viscosity value as it is shown in Figure 7. This is because the increasing of the droplet size of the nanoemulgel causing an increase in the viscosity of the preparations during storage. While, the viscosity on the emulgel during 12 weeks of storage decreased, this is associated with phase separation.

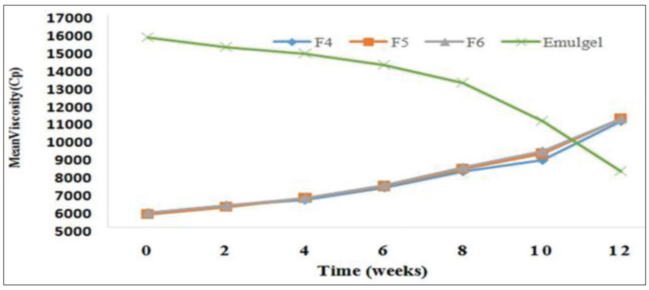

Figure 7: Effects of storage time on viscosity of sunscreen nanoemugels and emugel

There was no sign of phase separation for any sunscreen nanoemulgel formulations after the cycling test, indicating that the nanoemulgel would be stable under test conditions as is presented in Figure 8.

The average SPF value of all formulations of sunscreen nanoemulgels is shown in Table 5. It indicates that the average SPF value of nanoemulgels containing combination of grape seed oil and anisotriazine (F5 and F6) is higher than those containing grape seed oil only (F4).

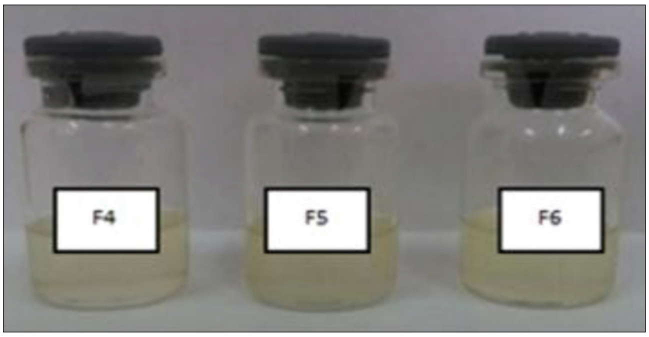

Figure 8: Cycling test result of sunscreen nanoemugels (F4, F5, and F6)

The transmission electron microscopy image of nanoemulgel is shown in Figure 9. The morphological analysis results of nanoemulgel were spherical in size <200 nm without any aggregation. This evaluation was performed on nanoemulgel containing 4% grape seed oil and anisotriazine 3.2% (F6) with the highest SPF value among all sunscreen nanoemulgel formulations.

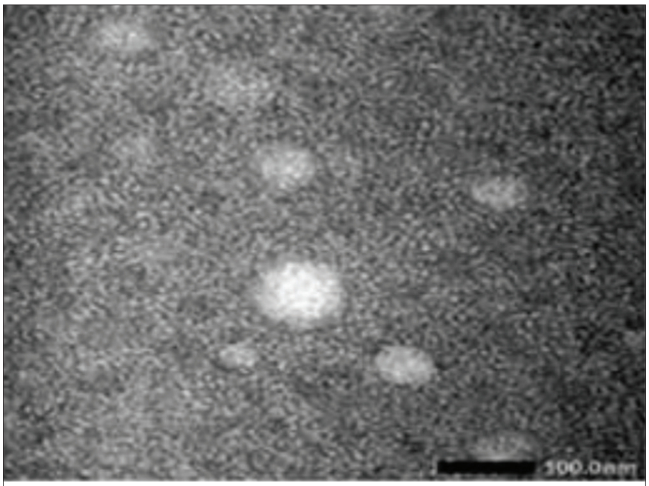

Figure 9: TEM image of sunscreen nanoemulgel containing 4% grape seed oil and 3.2% anisotriazine (F6)

Table 5: Average SPF value of sunscreen nanoemulgel and emulgel

Formula	SPF value						Average SPF value
	I	II	III	IV	V	VI	
F4 (nanoemulgel)	11.315	11.291	11.195	11.072	11.078	11.068	11.169 ± 0.113
F5 (nanoemulgel)	16.127	16.378	16.191	16.156	16.098	16.149	16.183 ± 0.100
F6 (nanoemulgel)	19.495	19.644	19.484	19.244	19.086	19.001	19.325 ± 0.232
Emulgel	11.615	11.804	11.946	11.997	12.006	12.110	11.913 ± 0.161

n = 6. SPF: Sun protection factor.

Discussion

The nanoemulsion in this study was prepared using Tween 80 as a surfactant with an HLB value of 15 (> 10) and sorbitol as cosurfactant to form an oil/water nanoemulsion [8]. Nanoemulsion with smaller droplet size was achieved using the magnetic stirrer and ultrasonicator. In the preparation of nanoemulsions, ultrasonication is used to produce mechanical vibrations and cavitation for the formation of stable nanoemulsion with small droplets [13]. Nanoemulsion containing grape seed oil and variations of anisotriazine is formulated by adding different concentrations of anisotriazine (2, 4, and 6%). A precipitate formation observed in the highest concentrations (6%) of anisotriazine, thus, the nanoemulsions with 2 and 4% were selected for the preparation of nanoemulgel, in which there was no precipitation formation after 12 weeks from the preparation time.

Nanoemulgel with transparent appearance is obtained by adding a gel solution to nanoemulsion in a ratio of 4:1. The mean droplet size of all nanoemulgel formulations was <200 nm. Nanoemulgel containing 4% grape seed oil and 3.2% anisotriazine (F6) had a droplet size of 187.5 nm. The droplet size of nanoemulgel in the range of 20–200 nm could be obtained using magnetic stirrer and ultrasonicator, which leads to a substantial reduction of the droplet size. The cost of this equipment was cheaper than other high-energy equipment and more flexible on surfactant selection than low-energy emulsification method [23], [24]. The physical stability evaluation results of the nanoemulgel which showed more stable compared to emulgel, this can be explained that nanoemulgel prepared with high-energy emulsification which results in a smaller droplet size but the emulgel prepared by mechanical agitation which results in a larger droplet size [20].

A combination of grape seed oil and anisotriazine provides higher SPF values due to the synergistic effect of the combination in the formulation of sunscreen nanoemulgel. In addition, nanoemulgel has a smaller particle size so that it will absorb more UVB rays which results in a higher SPF value. This results appropriate with previous research that a high SPF sunscreen product could be obtained by nanotechnology [6].

Conclusion

The sunscreen nanoemulgel formulation containing 4% grape seed oil and 3.2% anisotriazine was more stable compared with emulgel formulation. The SPF value of this nanoemulgel formulation is higher than nanoemulgel formulation without anisotriazine and emulgel.

References

1. Parkin DM, Mesher D, Sasieni P. 13. Cancers attributable to solar (ultraviolet) radiation exposure in the UK in 2010. Br J Cancer. 2011;105(2):S66-9. https://doi.org/10.1038/bjc.2011.486 PMid:22158324

2. D'Orazio J, Jarrett S, Amaro-Ortiz A, Scott T. UV Radiation and the skin. Int J Mol Sci. 2013;14(6):12222-48. https://doi.org/10.3390/ijms140612222 PMid:23749111
3. Pandel R, Poljšak B, Godic A, Dahmane R. Skin photoaging and the role of antioxidants in its prevention. ISRN Dermatol. 2013;2013:930164. https://doi.org/10.1155/2013/930164 PMid:24159392
4. Green AC, Williams GM, Logan V, Strutton GM. Reduced melanoma after regular sunscreen use: Randomized trial follow-up. J Clin Oncol. 2011;29(3):257-63. https://doi.org/10.1200/jco.2010.28.7078 PMid:21135266
5. Heurung AR, Raju SI, Warshaw EM. Adverse reactions to sunscreen agents: Epidemiology, responsible irritants and allergens, clinical characteristics, and management. Dermatitis. 2014;25(6):289-326. https://doi.org/10.1097/der.0000000000000079 PMid:25384223
6. Arianto A, Cella G, Bangun H. Preparation and evaluation of sunscreen nanoemulsions with synergistic efficacy on SPF by combination of soybean oil, avobenzone, and octyl methoxycinnamate. Open Access Maced J Med Sci. 2019;7(17):2751-6. https://doi.org/10.3889/oamjms.2019.745 PMid:31844431
7. Limsuwan T, Amnuikit T. Effect of grape seed extract in sunscreen lotion on sun protection factor (SPF) determined by *in vitro* method. In: Proceedings of the 6th International Conference on Bioinformatics and Biomedical Science. United States: ACM Press; 2017. https://doi.org/10.1145/3121138.3121192
8. Debnath S, Satayanarayana KVG, Kumar GV. Nanoemulsion-a method to improve the solubility of lipophilic drugs. PHARMANEST Int J Adv Pharm Sci. 2011;2(2-3):72-83.
9. Shinagawa FB, de Santana FC, Torres LR, Mancini-Filho J. Grape seed oil: A potential functional food? Food Sci Technol. 2015;35(3):399-406. https://doi.org/10.1590/1678-457x.6826
10. Garavaglia J, Markoski MM, Oliveira A, Marcadenti A. Grape seed oil compounds: Biological and chemical actions for health. Nutr Metab Insights. 2016;9:59-64. https://doi.org/10.4137/nmi.s32910
11. Goswami PK, Mayuri S, Rashmi S. Natural sunscreen agents: A review. SAJP. 2013;2(6):458-63.
12. Basera K, Bhatt G, Kothiyal P, Gupta P. Nanoemulgel: A novel formulation approach for topical delivery of hydrophobic drugs. WJPPS. 2015;4(10):1872-6.
13. Delmas T, Piraux H, Couffin AC, Texier I, Vinet F, Poulin P, *et al*. How to prepare and stabilize very small nanoemulsions. Langmuir. 2011;27(5):1683-92. https://doi.org/10.1021/la104221q
14. Koroleva MY, Yurtov EV. Nanoemulsions: The properties, methods of preparation and promising applications. Russ Chem Rev. 2012;81(1):21-43. https://doi.org/10.1070/rc2012v081n01abeh004219
15. Estanqueiro M, Conceição J, Amaral MH, Santos D, Silva JB, Lobo JM. Characterization and stability studies of emulsion systems containing pumice. Braz J Pharm Sci. 2014;50(2):361-9. https://doi.org/10.1590/s1984-82502014000200016
16. Jain A, Gautam SP, Gupta Y, Khambete H, Jain S. Development and characterization of ketoconazole emulgel for topical drug delivery. Der Pharm Sin. 2010;1(3):221-31.
17. Iradhati AH, Jufri M. Formulation and physical stability test of griseofulvin microemulsion gel. Int J Appl Pharm. 2017;9:23. https://doi.org/10.22159/ijap.2017.v9s1.22_27
18. Dutra EA, da Costa e Oliveira DA, Kedor-Hackmann ER, Santoro MI. Determination of sun protection factor (SPF) of sunscreens by ultraviolet spectrophotometry. Rev Bras Ciên Farma. 2004;40(3):381-5. https://doi.org/10.1590/s1516-93322004000300014
19. Sayre RM, Agin PP, LeVee GJ, Marlowe E. A comparison of *in vivo* and *in vitro* testing of sunscreening formulas. Photochem Photobiol. 1979;29(3):559-66. https://doi.org/10.1111/j.1751-1097.1979.tb07090.x PMid:441130
20. Shekar MC, Chary BB, Srinivas S, Kumar BR, Mahendrakar MD, Varma MV. Effect of ultrasonication on stability of oil in water emulsions. Int J Drug Deliv. 2011;3(1):133-40. https://doi.org/10.5138/ijdd.2010.0975.0215.03063
21. Chikakane K, Takahashi H. Measurement of skin pH and its significance in cutaneous diseases. Clin Dermatol. 1995;13(4):299-306. https://doi.org/10.1016/0738-081x(95)00076-r PMid:8665438
22. Bernardi DS, Pereira TA, Maciel NR, Bortoloto J, Viera GS, Oliveira GC, *et al*. Formation and stability of oil-in-water nanoemulsions containing rice bran oil: *In vitro* and *in vivo* assessments. J Nanobiotechnol. 2011;9(1):44. https://doi.org/10.1186/1477-3155-9-44 PMid:21952107
23. Yukuyama MN, Ghisleni DD, Pinto TJ, Bou-Chacra NA. Nanoemulsion: Process selection and application in cosmetics-a review. Int J Cosmet Sci. 2015;38(1):13-24. https://doi.org/10.1111/ics.12260 PMid:26171789
24. Mehmood T, Ahmed A, Ahmed Z, Ahmad MS. Optimization of soya lecithin and Tween 80 based novel Vitamin D nanoemulsions prepared by ultrasonication using response surface methodology. Food Chem. 2019;289:664-70. https://doi.org/10.1016/j.foodchem.2019.03.112