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 nanoeumulsions 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 nanoeumulgels 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 variations 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 absorber 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
eoil 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., Spain),
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
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
prepare 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 S-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 \left( \frac{\sum_{\lambda} EE(\lambda) \times (I(\lambda) \times Abs(\lambda))}{290} \right)

Where: CF: Correction factor (=10); EE:
Erythemal effect spectrum; I: Solar intensity spectrum;
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  | 5  |
| Tween 80         | 25                     | 26 | 27 | 27 |
| Sorbitol         | 35                     | 34 | 33 | 33 |
| Methylparaben    | 0.1                    | 0.1| 0.1| 0.1|
| Propylparaben    | 0.02                   | 0.02| 0.02| 0.02|
| Distilled water  | to 100                 | 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.

The composition of nanoemulgels containing grape seed oil and anisotriazine is presented in Table 2.

Table 2: Composition of grape seed oil and anisotriazine nanoemulgels

| Ingredients      | Quantity of 100 mL (%) | F4 | F5 | F6 |
|------------------|------------------------|----|----|----|
| Grape seed oil   | 5                      | 5  | 5  | 5  |
| Anisotriazine    | 4                      |    |    |    |
| Tween 80         | 27                     | 27 | 27 | 27 |
| Sorbitol         | 33                     | 33 | 33 | 33 |
| Methylparaben    | 0.10                   | 0.10| 0.10| 0.10|
| Propylparaben    | 0.02                   | 0.02| 0.02| 0.02|
| Distilled water  | to 100                 | 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.

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 irri
table 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   | 7.03 ± 0.06| 7.06 ± 0.11| 7.20 ± 0.10| 7.03 ± 0.06|
| F5   | 6.96 ± 0.06| 6.93 ± 0.06| 6.93 ± 0.06| 6.86 ± 0.06|
| F6   | 6.88 ± 0.06| 6.83 ± 0.06| 6.83 ± 0.06| 6.73 ± 0.06|
| Emulgel | 6.66 ± 0.06| 6.60 ± 0.10| 6.56 ± 0.11| 6.43 ± 0.05|

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

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.

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.

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).
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.

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 11.076 | 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.

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