INTRODUCTION

The skin forms a protective barrier over the body's surface, which is continuously exposed to air, solar radiation, and pollutants that can stimulate the formation of free radicals [1]. The skin's sebum and cell membrane lipids contain an unsaturated fatty acids double chain, which is the main target of free radicals [2]. Further, the skin's protective capability is limited, and problems arise when environmental exposure exceeds the skin's normal defense potential [3]. The epidermis is the first protective layer of the skin against oxidative damage. It contains a higher antioxidant capacity than the dermis, thereby providing greater defense [4]. Oxidative stress occurs when free radicals and the active substance within a system exceed the system's ability to neutralize and eliminate free radicals [5].

Free radicals are also involved in the physiological activity of the body, for example, helping to maintain homeostasis at the cellular level in normal tissues [6]. However, free radicals can be produced in excess through cigarette smoke, air pollution, ultraviolet (UV) rays, and other means [7], with UV radiation being responsible for up to 80% of the oxidative stress on the skin [1]. Antioxidants prevent the tissue damage caused by free radicals by preventing the excess formation of free radicals. Antioxidants are molecules that are stable enough to donate electrons to free radicals to neutralize them, thereby reducing their ability to cause damage. Antioxidants can be divided into two categories, namely, enzymatic and nonenzymatic. Nonenzymatic antioxidants such as Vitamin E, Vitamin C, and β-carotene are not produced by the body [8]. The use of topical antioxidants enhances antioxidant availability by providing high concentrations of antioxidant compounds to the skin. This increases the antioxidant defenses of the skin and reduces the harmful effects of free radicals and oxidative stress, as well as helping to prevent skin aging [9].

Vitamin E has been widely used as a fat-soluble natural antioxidant that can be divided into two classes, namely tocopherols and tocotrienols. Researchers have suggested that tocotrienol has an antioxidant potential higher than that of tocopherols. Previous studies have shown that δ-α-tocotrienol has a 40-60 times higher antioxidant potency than δ-α-tocopherol [10]. If the skin is exposed to oxidative stress generated by UV radiation, increasing the antioxidant content can prove sufficient to combat that oxidative stress [11]. Nowadays, nanotechnology has become an increasingly popular technology, with one potential use being nanoeumulsion. Nanoeumulsion can be used as a potential carrier for drugs or cosmetics, and it can serve to optimize the dispersion of active compounds within certain skin layers. This study aims to measure the antioxidant activity of nanoeumulsion gel formulations containing tocotrienol. It will also evaluate and test the physical stability of nanoeumulsion gel formulations containing tocotrienol.

MATERIALS AND METHODS

Experimental

Materials
Tocotrienols (Gold-TriE™, Malaysia), Vitamin C (CSPC Weisheng Pharmaceutical), propylene glycol (Dow Chemical Co.), 96% ethanol (Indo Akadatama), oleic acid (Kao Corporation), carborner (Labrotig), triethanolamine (Brataco, Indonesia), 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Sigma-Aldrich), Tween 80 (Kao Corporation), methyl paraben (Brataco, Indonesia), propyl paraben (Brataco, Indonesia), aquadest (Brataco, Indonesia), methanol p.a. (Merck), and butyldihydroxytoluene (BHT) (Brataco, Indonesia).

The instruments used in the study included an analytical scale (Adam AFA - 210 LC, USA), a homogenizer (Ika T25 Digital Ultra-Turrax, Germany), a homogenizer (Multipin, Malaysia), a pH meter (Eitech pH510, Singapore), a pycnometer, a Brookfield viscometer (Brookfield Engineering Laboratories, Inc., USA), a “centrifugator” (Kubota 5100, Japan), a Zetasizer (Malvern, United States), transmission electron microscope (TEM) (JEOL, JEM-1010), a refrigerator (Toshiba, Japan), an oven (Memmert, Germany), a UV-visible spectrophotometer (Shimadzu UV-1601, Japan), a thermometer, and glass tools.

Designing the pseudo-ternary phase diagram

The water titration method was used to determine the phase composition of the appropriate oil, water, surfactant, and cosurfactant.
to form the nanoemulsion by designing pseudo-ternary phase diagrams. Oleic acid represented the oil phase, aquaeast - the water phase, Tween 80 - the surfactant, and 96% ethanol and propylene glycol as co-surfactants. For each phase diagram, a mixture of oil, surfactant, and co-surfactant (Smix) at a certain ratio was prepared thoroughly from 1:9 to 9:1 in different vials. Nine different ratios of Smix namely 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, and 9:1, were dripped with some aquaeast to make the mixture become cloudy, and then the volume of water that dripped was noted. The pseudo-ternary phase diagrams were formed using the Chemix School version 3.60 program.

NANOEMULSION GEL FORMULATION

The nanoemulsion gel was made by adding a nanoemulsion to the gel base. First, the nanoemulsion was made according to three different formulations, each with the same concentration of tocotrienol, namely 0.275% (w/w). An oleic acid concentration of 4%, 5%, or 6% (w/w). Tween 80, and aquaeast were added slowly, and the surfactant mixture was stirred until homogenization using a homogenizer at a speed of 5000 rpm on the heater and then the temperature was set to 40±2°C. The temperature was lowered to 27±2°C and then the mixture cooled to room temperature (27±2°C). In a different container, oleic acid, tocotrienol, and BHT as the oil phase were mixed and stirred until homogenous. Then, the oil phase was mixed into the aquaeast and surfactant mixture while constant stirring continued. Another container; methyl paraben and propyl paraben were dissolved in propylene glycol. A mixture of propylene glycol and 96% ethanol was added slowly into the oil phase and surfactant mixture and then stirred until homogenous at a speed of 5000 rpm for 5 minutes to form a clear nanoemulsion. The gel was formed by a carborner and triethanolamine, which were stirred homogeneously with a homogenizer at a speed of 300 rpm for 5 minutes. Next, the nanoemulsion mixture was mixed with the gel and then stirred at a speed of 500 rpm for 7 minutes. Gel base formula and the tocotrienol nanoemulsion gel formula are described in Tables 1 and 2.

EVALUATION AND PHYSICAL STABILITY TEST OF THE NANOEMULSION GEL

An organoleptic observation pH measurement, globule size measurement, droplet morphology measurement, and viscosity measurement were all conducted to evaluate the nanoemulsion gel. The globule size was calculated using a particle size analyzer (PSA), whereas the droplet morphology was identified by means of TEM. The measurement of the viscosity and flow properties of the nanoemulsion gel was performed using a Brookfield viscometer. The preparation was stored at a low temperature (4±2°C), room temperature (27±2°C), and high temperature (40±2°C) for 8 weeks. The viscosity measurement and globule size measurement were conducted at week 0 and 8. Further, in terms of the cycling test, the nanoemulsion gel samples were stored at 4°C for 24 hrs and then transferred into an oven at a temperature of 40±2°C for 4 hrs (i.e., one cycle).

ANTIOXIDANT ACTIVITY MEASUREMENT USING THE DPPH METHOD

The free radical scavenging activity of the nanoemulsion gel containing tocotrienol was measured using 1,1-diphenyl-2-picrylhydrazyl (DPPH). A solution of 100 ppm of DPPH in methanol was prepared. Next, 1 ml of the DPPH solution was added to 2 ml of methanol and 1 ml of nanoemulsion gel at different concentrations (3000, 5000, 7000, 10,000, 12,000, and 15,000 μg/ml). Each of these concentrations was wrapped in aluminum foil and stored in a dark room at a temperature of 37°C for 30 minutes. Then, its absorbance was measured using a UV-VIS spectrophotometer at the maximum wavelength.

RESULTS

Designing the pseudo-ternary phase diagrams

The diagrams had three axes, with the first axis being the water phase, the second axis being the oil phase, and the third axis being the mixture of surfactants and co-surfactants.

Fig. 1 demonstrates the formation regions with the nanoemulsion and it showed as the red area. The red area was considered to indicate an easy flowing nanoemulsion formulation, whereas the transparent area was seen by visual observation.

PREPARATION OF THE TOCOTRIENOL NANOEMULSION GEL

The best formulation of the nanoemulsion gel was used to make the nanoemulsion gel. The best formula chosen based on its small globule size and stability. The best formulation was Formula 1, which had the smallest globule size of 219.2 nm and the greatest stability among the other formulas. Formula 2 had a globule size of 273.8 nm, and Formula 3 first exhibited separation after a few weeks.

EVALUATION OF THE NANOEMULSION GEL

The nanoemulsion gel at week 0 appeared yellow in color (Pantone 100 CS), viscous, and homogeneous, and no phase separation occurred. The nanoemulsion gel at week 8 at room temperature also appeared yellow in color (Pantone 100 CS), viscous, and homogeneous, and again no phase separation occurred. This showed that the nanoemulsion gel remained stable after 8 weeks. The results of the pH measurement of the nanoemulsion gel (Formula 1) indicated a pH value of 6.12. Furthermore, the globule size distribution was also measured. In this study, the globule size distribution of the nanoemulsion was measured using a PSA. The nanoemulsion globule size was measured at week 0.

Table 1: Gel base formula

| Material   | Concentration (%) w/w |
|------------|-----------------------|
| Carbomer   | 1                     |
| Triethanolamine | 1                  |
| Aquaeast   | Ad 100                |

Table 2: Tocotrienol nanoemulsion gel formula

| Material      | Concentration (%) w/w |
|---------------|-----------------------|
| Tocotrienol   | 0.275                 |
| Oleic acid   | 4                     |
| Tween 80     | 25                    |
| 96% ethanol  | 10                    |
| Propylene glycol | 5                  |
| Methyl paraben | 0.2                 |
| Propyl paraben | 0.1                 |
| BHT          | 0.05                  |
| Gel base     | 20                    |
| Demineralized water | 35               |

BHT: Butylhydroxytoluene
and week 8. The nanoemulsion globule size at week 0 was 219.2 nm, whereas at week 8, the globule size had increased to 261.7 nm. The nanoemulsion gel globule size was 596.4 nm. The zeta potentials of the nanoemulsion and the nanoemulsion gel were −30 mV and −27.1 mV, respectively (Table 3).

In addition, a gel viscosity test was conducted. The measurement of the gel viscosity was performed at week 0 and week 8. The viscosity test showed that the nanoemulsion gel had 284.84 poise and 322.19 poise at week 0 and week 8, respectively. It can be seen that the viscosity of the nanoemulsion gel increased over the 8 weeks. The nanoemulsion gel exhibited plastic flow properties. The nanoemulsion morphology can be seen in Fig. 2.

**Physical stability test of the nanoemulsion gel**

A physical stability test was carried out by observing the nanoemulsion gel in storage conditions at a variety of different temperatures, namely, room temperature (27±2°C), high temperature (40±2°C), and low temperature (4±2°C), for 8 weeks. The physical stability was evaluated using the centrifugation test and the cycling test. The observation of the organoleptic and pH at low-temperature storage (4±2°C), room temperature storage (27±2°C), and high-temperature storage (40±2°C) for 8 weeks showed that there was no phase separation in the nanoemulsion gel. However, the color changed and became more brownish in the high-temperature storage (40±2°C). A mechanical test was conducted to determine the stability of the nanoemulsion gel and identify the effects of gravitation force using a centrifugator at a speed of 3800 rpm for 5 hrs. The results were equivalent to the effects of gravity for 1 year. The results showed that the nanoemulsion gel remained homogeneous and no phase separation occurred. A cycling test was conducted by storing the samples at a low temperature (4±2°C) for 24 hrs and then transferring the samples into storage at a high temperature (40±2°C) for 24 hrs. The procedure was calculated as one cycle. The cycling test was carried out in six cycles in a row to determine the condition of the samples in extreme temperatures and observe the formation of crystals. The results obtained were still homogeneous, no phase separation occurred, and there was no crystal formation.

**Measurement of antioxidant activity using the DPPH method**

The measurement of the antioxidant activity of four samples was performed. The samples were Vitamin C, as a positive control, oil tocotrienol, a nanoemulsion of tocotrienol, and a tocotrienol nanoemulsion gel at week 0 and week 8. The IC_{50} values are presented in Table 4.

**DISCUSSION**

The oil phases used in the tocotrienol nanoemulsion were tocotrienol oil and oleic acid. The percentages of the oleic acid used in the manufacture of the nanoemulsion were 4%, 5%, and 6% in the three formulas. The formula with the addition of 6% oleic acid showed instability at 2 weeks. The nanoemulsion exhibited separation. This was caused by the lack of the surfactant concentration necessary to stabilize the nanoemulsion since the amount of oil was not balanced with a surfactant and the surfactant was insufficient to envelop the oil, which could lead to breaking/cracking. Oleic acid was selected as the oil phase because it can increase the fluidity of the stratum corneum and induce the permeation pathway to the stratum corneum [12]. It also acts as an emulsifier.

The utilized cosurfactants were 96% ethanol and propylene glycol. Short-medium chain alcohols such as 96% ethanol and propylene glycol are usually added as cosurfactants to reduce the interfacial tension and improve the fluidity of the interface fluid. Short-medium chain alcohols also increase the mobility of the hydrocarbon tail and allow greater penetration of the oil into the site of action [13]. In addition, as well as being a cosurfactant, at a concentration of 2-5%, propylene glycol can be used as an antibacterial activity enhancer of methyl paraben and propyl paraben. Further, BHT can be added to the preparation as an antioxidant. Antioxidants also need to be added to protect the nanoemulsion from oxidation. BHT can be dissolved in oil, which means that it could be blended into the oil phase of the nanoemulsion. In the evaluation of the nanoemulsion gel, the pH value was found to be 6.12.

In terms of the globule size distribution and zeta potential, changes in the size of the nanoemulsion globules can occur due to flocculation, which is a merging of globules that leads to coalescence (i.e., increased globule size). When the nanoemulsion was stored for a long period of time, the size of the globules increased. The zeta potential is an indicator of the stability of the nanoemulsion because it demonstrates the electrostatic repulsion between adjacent particles. If the value of the zeta potential is high, it will prevent the aggregation of the nanoemulsion due to the repelling force among the particles. As a nonionic surfactant, Tween 80 can maintain the stability of the single layer nanoemulsion. As a standard, zeta potential values above ±30 mV indicate good stability in a nanoemulsion [14]. The zeta potential value obtained for the nanoemulsion gel was −27.1 mV. In the nanoemulsion gel, the globule size increased to a greater extent than in the nanoemulsion, which led to the nanoemulsion gel becoming less stable than the nanoemulsion.

The pH value reduction indicated by the stability test could result in a decrease in the zeta potential (i.e., could become less negative) and an increase in the speed of flocculation [15].

With regard to the stability test, the decrease in the pH observed during the stability test indicated the presence of H^+ ions that were released since there was a tendency for the material in the nanoemulsion gel to be oxidized against the temperature and oxygen so that it released H^+ ions. The free H^+ concentration in the nanoemulsion gel caused it to become more acidic, so there was a decrease in the pH value of

![Fig. 2: Morphology of the nanoemulsion (transmission electron microscope magnification ×30,000)](image-url)
the nanoemulsion gel. A significant decrease in the pH was seen at a high temperature (40°C±2°C) because at higher temperatures, the ionization reaction becomes faster; hence, the pH reduction at a high temperature was higher than that seen at room temperature and low temperature (4°C±2°C).

In terms of the antioxidant activity of the nanoemulsion gel, antioxidant compounds with IC₅₀ values <50 ppm are classified as very powerful antioxidants, those with IC₅₀ values between 50 and 100 ppm are classed as powerful antioxidants, those with IC₅₀ values ranging between 100 and 150 ppm are classified as antioxidants, and those with IC₅₀ values ranging between 150 and 200 ppm are classed as weak antioxidants [16,17]. There was a decrease in antioxidant activity during storage that was caused by Vitamin E since the antioxidant was sensitive to temperature, light, and oxygen. It allowed a reduction in the antioxidant activity of the tocotrienol nanoemulsion.

CONCLUSION

The nanoemulsion gel formulation retained antioxidant activity and was physically stable for 8 weeks. The characteristics of nanoemulsion gel were yellow in color (Pantone 100 CS), viscous, homogeneous with no phase separation occurred, and no crystal formation occurred after the cycling test had been performed.

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