Griseofulvin is an effective antifungal drug for several species of fungi, such as Microsporum, Epidermophyton, and Trichophyton [1]. Griseofulvin is practically insoluble in water and is a biopharmaceutics classification system Class II drug, which means that it has a low solubility and a high permeability [2]. Due to its low water solubility, griseofulvin has a low dissolution rate, which leads to low drug bioavailability. Low griseofulvin absorption can be increased by formulating it as a microemulsion [3], which led to the selection of griseofulvin microemulsion gel preparation as the topic for this study.

Griseofulvin may cause some systemic side effects if given orally over the long term [4]. Side effects that may arise include proteinuria, nephrosis, leukopenia, hepatitis, clotting disorders, liver enzyme elevation, hyperbilirubinemia, and bleeding in the digestive tract [5]. To avoid these adverse effects, a griseofulvin microemulsion gel preparation for topical use was formulated to overcome these problems.

METHODS

Pseudoternary phase diagram

Constructing a pseudoternary phase diagram helps to determine whether the formula will provide a good microemulsion preparation. The pseudoternary phase diagram shows the concentrations of water, oil, and surfactants-cosurfactants to be used in formulations to produce microemulsions. A pseudoternary phase diagram is done using the water titration method. A mixture of surfactant and cosurfactant (Tween 80 and 96% ethanol 96%, with ratio 5:4) and oil phase (oleic acid) were mixed at the following ratios: 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2 and 9:1. The mixture was added slowly to Aquadest and shaken to form a clear liquid, which indicated that a microemulsion had formed. The pseudoternary phase diagram was made from the results of the experiments obtained using CHEMIX School version 3.60.

Preliminary experiments

Preliminary experiments were performed to obtain the concentrations of oleic acid, Tween 80, and 96% ethanol that produced the best griseofulvin microemulsion formula. The concentrations of oleic acid used were 5%, 7%, and 9%, the concentrations of Tween 80 used were 20%, 25%, 30%, and 35%, and the ethanol concentration used was 20% and 25%. The best microemulsion formulation provided a clear solution.

Griseofulvin microemulsion gels

The microemulsions were made by mixing the ingredients into the oil phase or the water phase separately. The Aquadest and Tween 80 were mixed and heated to a temperature of 40°C. Amount of griseofulvin was dissolved into oleic acid and then mixed with the Aquadest and Tween 80 mixture using a homogenizer at 5000 rpm. The 96% ethanol was then added to the oil and water phase mixture, while continuously stirring with the homogenizer. The microemulsions were then allowed to stabilize for 24 hrs.

The gel base was made in a separate container. Carbopol 940 was dissolved in water, while during stirred, and triethanolamine was slowly added. The Carbopol 940 and triethanolamine mixture was then stirred using a homogenizer at 2000 rpm to form a gel base, and allowed to stabilize for 24 hrs. After sterilization, the microemulsion was then slowly added to the gel base, while stirring using a homogenizer at a rate of 3000 rpm to form a griseofulvin microemulsion gel.

Evaluation of the griseofulvin microemulsion gel preparations

The evaluations test was performed on the griseofulvin microemulsion gel preparations, as well as globule size distribution, pH, viscosity, and rheological measurements. Organoleptic observations were reseased to evaluate the form, color, odor, and clarity of the preparations. These observations were done every 2 weeks for 8 weeks. The globule size distribution was done using a particle size analyzer tool at weeks 0 and 8. The pH of the preparation was measured using a pH meter, and the measurements were performed 3 times that had been calibrated with a buffer solution at pH 4 and 7. The desired preparation was within the pH range of 5.5-5.9. The pH measurements were performed weekly for 8 weeks.

The viscosity and rheological measurements were performed using a Brookfield viscometer. Measurements were made at room temperature (28°C) at weeks 0 and 8.

Physical stability tests of griseofulvin microemulsion gel

The following physical stability tests were performed on the griseofulvin microemulsion gel preparations: Storage test at 4°C±2°C, 25°C±2°C, and 40°C±2°C.
storage test at 25°C±2°C, storage test at 40°C±2°C, and mechanical test (centrifugation). The cycling test involved up to 6 cycles. Each cycle consisted of storing the preparations at 4°C±2°C for 24 hrs and then at 40°C±2°C for 24 hrs this experiment was repeated 3 times.

The storage test at 4°C±2°C was done by storing the microemulsion preparation at 4°C±2°C for 8 weeks. Organoleptic observations of the physical conditions and measurements of the pH were performed every 2 weeks. The storage tests at 25°C±2°C and 40°C±2°C were carried out in the same manner.

The mechanical test was carried out using centrifugation. Samples of the microemulsion preparation were centrifuged at 3800 rpm for 5 hrs. This treatment is equivalent to the gravitational effect for 1 year. Organoleptic observations were done on the physical condition of the preparation before and after centrifugation. This experiment was performed 3 times.

RESULTS AND DISCUSSION

Pseudoternary phase diagram

The pseudoternary phase diagram is shown in Fig. 1. It is shown that increased levels of oleic acid and decreased levels of surfactant-cosurfactant mixture required less moisture to produce the microemulsion. For example, for a ratio of oleic acid and surfactant-cosurfactant of 1:9, the water added was proportional to the amount of oleic acid and mixed surfactants. However, for a ratio of oleic acid and surfactant-cosurfactant 1:9, the water added only 1/10 of the total amount of the mixture. This can be due to the high concentration of oleic acid; since the outer phase is the oil phase, the addition of water in large quantities causes unstable microemulsions.

Preliminary experiments

Preliminary experiments as shown in Table 1, were conducted to determine the best concentrations of oil, surfactants, and cosurfactants to be used in the griseofulvin microemulsion gel formula. The preliminary results showed that as the level of the oleic acid increased. This is because large oil content requires more hydrophilic-lipophilic balance (HLB) to make the emulsion [6]. In addition, the more the oil phase is used, the greater the volume of oil that is formed into globules, thereby reducing the surfactant levels [7]. Thus, the more oleic acid used in the formulation, the greater the need for HLB and the amount of surfactant required to form the micelles, which means that the required levels of Tween 80 and 96% ethanol 96% are also greater. The inner phase content of oleic acid also makes the emulsion more unstable because of the greater interface tension, which requires increased levels of Tween 80 and 96% ethanol (Table 2).

From the experimental results, 5% oleic acid, 25% Tween 80, and 20% ethanol were selected to prepare the griseofulvin microemulsion gel formula.

Griseofulvin microemulsion gels

The oleic acid content 5% give a stable microemulsion with the lowest levels of Tween 80 and 96% ethanol. The levels of Tween 80 and 96% ethanol were 25% and 20%, respectively. Using low levels of Tween 80 and 96% ethanol in the microemulsion were intended to reduce the likelihood of skin irritation since both Tween 80 and 96% ethanol can irritate the skin [5,8]. Ethanol 96% can also cause dermatitis, especially in patients with aldehyde dehydrogenase deficiency [8]. The gel base comprised of 1% Carbopol 940, 1% triethanolamine, and Aquadest. The gel base was made to stand for 24 hrs before mixing with the microemulsions to remove any bubbles trapped in the gel.

| Formula | Oleic acid (%) | Tween 80 (%) | 96% ethanol (%) | Aquadest (%) | Result |
|---------|----------------|--------------|-----------------|--------------|--------|
| F1      | 5              | 20           | 20              | 55           | Turbid |
|         | 25             | 20           | 20              | 50           | Clear  |
|         | 30             | 25           | 25              | 45           | Clear  |
|         | 35             | 25           | 25              | 40           | Clear  |
| F2      | 7              | 20           | 20              | 53           | Turbid |
|         | 25             | 20           | 20              | 48           | Clear  |
|         | 30             | 25           | 25              | 43           | Clear  |
|         | 35             | 30           | 20              | 38           | Clear  |
| F3      | 9              | 20           | 20              | 33           | Clear  |
|         | 25             | 20           | 20              | 36           | Clear  |

Fig. 1: Pseudoternary phase diagram of microemulsions [3]
Organoleptic evaluation
The griseofulvin microemulsion had a transparent yellow color and smelled of alcohol. Griseofulvin microemulsion gels had a yellow slightly turbid, and alcohol odor. The microemulsions did not show phase separation before and after the addition of the gel base, which meant that the microemulsion was stable. When applied to the skin, the microemulsion gel preparations felt cold because of the high 96% ethanol concentration.

Measurement of the globule size distribution
The measurements of the globule size were done using a Zetasizer particle size analyzer software version 6.20 (Malvern). At week 0, the globule size was 158.0 nm (Table 3), which did not meet the criteria for microemulsion globule size, which is 1-100 nm. The size of microemulsion globule was large because the concentration of Tween 80 and 96% ethanol was quite low. The higher the surfactant-cosurfactant concentration, the smaller the size of the globule will be because the internal phase is immobilized in the surfactant [9]. Note that the microemulsion preparation still looked clear despite having a globule size that was >100 nm because an emulsion with globules smaller than 200 nm has a transparent appearance [10]. The addition of the gel base increases the size of the globule in the microemulsion.

The results of the globule size test at week 8 showed a decrease in globule size. However, this was followed by a decrease in the size of the globule. This can be seen from the increased polydispersity index of the microemulsion and the microemulsion gel, and the peaks of the globule size of the microemulsion gel were more than one.

The increase in globule size followed by the reduced uniformity of the globular sizes in the microemulsion and the microemulsion gel preparations can be due to the flocculation of large particles. A nanoparticle preparation with a potential zeta >+30 mV and smaller than ~30 mV exhibits good stability, while preparations with small potential zeta values indicate rapid particle aggregation due to van der Waals forces [6,11]. It can be concluded that the microemulsion preparation was more stable than the microemulsion gel preparation.

pH measurement
The pH of the microemulsion and the microemulsion gel was 5.52 because there was no change in pH, which was due to the decrease in temperature [6]. Testing the pH of the preparation during storage at temperature 4°C±2°C for 8 weeks produced fluctuating results, which showed that the pH tended to decrease (Fig. 3). This decrease in pH can be due to the oxidation of the oleic acid.

Storage test at 4°C±2°C
After 8 weeks, the storage test results showed that the preparation was stable because there were no changes in color, odor, or syneresis, although the viscosity of the dosage increased, which was due to the preparation at a temperature of 4°C±2°C for 8 weeks produced fluctuating results, which showed that the pH tended to decrease (Fig. 3). This decrease in pH can be due to the oxidation of the oleic acid.

Storage test at 25°C±2°C
After 8 weeks, the storage test results showed that the preparation was stable because there were no changes in color, odor, or syneresis, although the viscosity of the dosage increased, which was due to the storage at temperature 4°C±2°C for 8 weeks produced fluctuating results, which showed that the pH tended to decrease (Fig. 3). This decrease in pH can be due to the oxidation of the oleic acid.

Storage test at 40°C±2°C
The results from the storage test after 8 weeks showed that the preparations were stable because there were no changes in color, odor, or syneresis (Fig. 5).

Mechanical test (centrifugation)
The mechanical test was performed to see the effect of gravity on the stability of the microemulsion gel preparation (Fig. 6). The preparation remains stable because there were no changes in color, odor, or syneresis.
was centrifuged at 3900 rpm for 5 h, which is equivalent to the gravitational force for a year. The test was conducted 3 times. The results of the centrifugation test on the griseofulvin microemulsion gel preparation showed no phase separation or microemulsion separation from the gel.

CONCLUSION

The best griseofulvin microemulsion gel formula consisted of 25% concentration of Tween 80 as a surfactant, 20% concentration of 96% ethanol as a cosurfactant, 5% concentration of oleic acid as the oil phase, and 20% concentration of gel base. The gel base contained 1% Carbopol 940 as the gelling agent and 1% triethanolamine as the pH regulator. The microemulsion preparation had a transparent yellow color, while the microemulsion gel preparation gave a yellow, but slightly turbid, color. The microemulsion and microemulsion gel preparations had a globule size of 158.0 nm and 226.0 nm, respectively. The results of the cycling test showed that the microemulsion gel preparation was stable against the gravitational effect. For future studies, an antifungal activity test should also be done to determine the effectiveness of the preparation.

REFERENCES

1. International Agency for Research on Cancer. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Vol. 79. Lyon, France: IARC Press; 2001. p. 75-89.
2. Tanaka Y, Waki R, Nagata S. Species differences in the dissolution and absorption of griseofulvin and albendazole, biopharmaceutics classification system class II drugs, in the gastrointestinal tract. Drug Metab Pharmacokinet 2013;28(6):485-90.
3. Aggarwal N, Goindi S, Khurana R. Formulation, characterization and evaluation of an optimized microemulsion formulation of griseofulvin for topical application. Colloids Surf B Biointerfaces 2013;105:158-66.
4. Moghimipour E, Salimi A, Hassanvand S. Permeability assessment of griseofulvin microemulsion through rat skin. Int J Pharm Chem Biol Sci 2013;3(4):1061-5.
5. U.S. National Library of Medicine. Griseofulvin-Griseofulvin Table. 2016. Available from: http://www.dailymed.nlm.nih.gov/dailymed/drugInfo.cfm?setid=6149c044-58bb-402c-a4b5-18b96dd9b3b9. [Last accessed on 2016 Jan 11].
6. Sinko PJ. Farmasi Fisika dan Ilmu Farmasetika. 5th ed. Jakarta: Buku Kedokteran EGC; 2006.
7. Maya L. Pembuatan sediaan mikroemulsi dari minyak buah merah (Pandanus conoideus). Skripsi. Depok: Universitas Indonesia; 2006.
8. Lachenmeier DW. Safety evaluation of topical applications of ethanol on the skin and inside the oral cavity. J Occup Med Toxicol 2008;3:26.
9. Sindre U, Pokharkar S, Modani S. Design and evaluation of microemulsion gel system of nadifloxacin. Indian J Pharm Sci 2012;74(3):237-47.
10. Kumar A, Kushwaha V, Sharma PK. Pharmaceutical microemulsion: Formulation, characterization and drug deliveries across skin. Int J Drug Dev Res 2014;6(1):1-21.
11. Júnior AA, Baldo JB. The behavior of zeta potential of silica suspensions. N J Glass Ceram 2014;4:29-37.