



# Development of the Forskolin Microemulsion Formula and its Irritation Test on Rabbits

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## ABSTRACT

**Objectives:** This study aimed to develop a microemulsion formula that can increase the solubility and stability of forskolin and its safety for topical use.

**Materials and Methods:** The materials used for the development of the microemulsion formula were triglyceride oil, non-ionic surfactants, and polyethylene glycol (PEG) for cosurfactants, which were selected on the basis of the results of the forskolin solubility test using high performance liquid chromatography (HPLC). The microemulsion was formulated by the phase titration method. Formula stability was determined by storage for 90 days in a refrigerator at room temperature, and an accelerated stability test was performed by determining globule size, forskolin concentration, and pH. The safety of using microemulsions was determined by skin irritation tests on albino rabbits.

**Results:** The optimum microemulsion formula consisted of Maisine<sup>®</sup> CC, polyoxyethylene sorbitan 20 (POE 20), and PEG 400 with a ratio of 4:25:5 w/v, which increased the solubility of forskolin the most, namely 2.19 mg mL<sup>-1</sup>. Based on globule size (<50 nm), forskolin concentration (2 mg.mL<sup>-1</sup>), and pH (6.0-6.35), the formula was stable in refrigerator storage and room temperature but unstable in the accelerated stability test (40 °C) starting on day 21. This optimum formula exhibits a primary irritation index (PII) of 0.11, which is categorized as feeble irritation and can be ignored.

**Conclusion:** The microemulsion prepared by the phase titration method containing Maisine<sup>®</sup> CC, POE 20, and PEG 400 (4:25:5, w/v) as a base and 0.2% forskolin was stable in refrigerator storage and at room temperature. This microemulsion is mild or negligible irritant with a PII: 0.11.

**Key words:** Microemulsion, forskolin, skin irritation test

## INTRODUCTION

A microemulsion is a colloidal dispersion composed of water, oil, and a mixture of cosurfactants (Smix) that spontaneously occurs.<sup>1</sup> Microemulsion is a delivery system that can function as a solubility enhancer,<sup>2,3</sup> penetration,<sup>4-7</sup> stability, and diffusion and can dissolve lipophilic and hydrophilic active pharmaceutical ingredients (API).<sup>8</sup>

Each microemulsion component has a specific function. Oil can increase the hydrophilic API partition coefficient in the skin and change the conformation of lipids in the *stratum corneum*.<sup>9</sup> In contrast, surfactants can reduce surface tension, increase API solubility, and increase skin penetration.<sup>10</sup> The cosurfactant

increases the density and membrane permeability of the microemulsion globule monolayer, which is formed together with the surfactant, so that the monolayer membrane does not leak easily.

Forskolin is a secondary metabolite found in the roots of *Coleus forskohlii* Briq. This plant is often found in India, Nepal, Burma, and Thailand.<sup>11</sup> One of the pharmacological effects of forskolin is its lipolytic activity, where forskolin can activate the adenylate cyclase enzyme in adipose cells to stimulate the formation of cyclic adenosine monophosphate (cAMP). *In vitro*, forskolin can induce lipolysis by increasing the concentration of cAMP and forming protein kinase A.<sup>12-14</sup> Organoleptically; forskolin is in the form of a white powder with a molecular weight of 410.5

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grams.mol<sup>-1</sup>.<sup>15</sup> The molecular structure of forskolin is shown in Figure 1. Forskolin is insoluble in water with  $\log p = 3.89$ <sup>15</sup> but undergoes hydrolysis and isomerization at temperatures above 50 °C.<sup>16</sup>

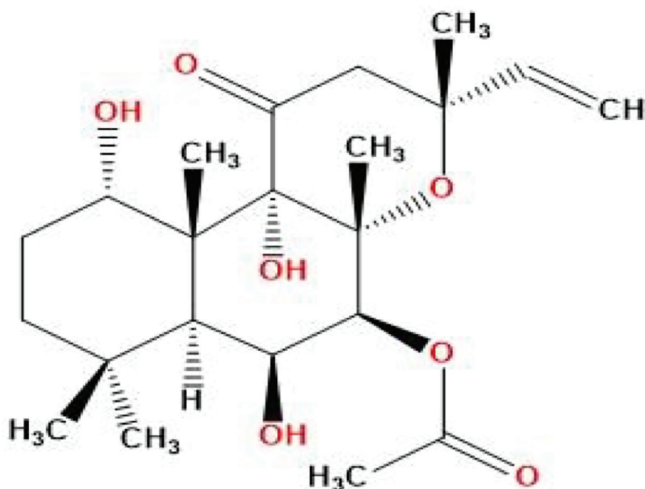


Figure 1. Molecular structure of forskolin

Forskolin is generally expressed in capsule dosage forms and is used orally as a slimming agent. The development of formulas in topical dosage forms can increase their usefulness. The microemulsion was chosen as a delivery system for forskolin because it can increase solubility by interacting with the microemulsion-forming material with forskolin. In addition, the microemulsion formulation uses low energy at room temperature, thereby avoiding the breakdown of forskolin, which is unstable at high temperatures.

Material selection can affect the success of microemulsion formulations. A high monoglyceride content in oil can increase the solubility of API with  $\log p > 2$ .<sup>17</sup> Acharya et al.<sup>2</sup> found that a microemulsion containing triglyceride oil can increase the solubility of aceclofenac. In this study, a microemulsion was developed based on the length of the carbon chain of triglyceride oil. The oil used is Maisine<sup>®</sup> CC, which is a long-chain triglyceride oil of oleic and linoleic unsaturated fatty acids (C18:1, C18:2), and medium-chain triglyceride (MCT), which is a medium-chain triglyceride oil of caproic, caprylic, capric, and lauric saturated fatty acids (C6–C12).<sup>18</sup>

One of the undesirable effects of microemulsions on the skin is their irritating properties due to high concentrations of surfactants. Wang et al.<sup>19</sup> found that a rotigotine microemulsion containing 25.2% Smix did not irritate the skin. Soliman et al.<sup>20</sup> also found that celecoxib microemulsion containing 45.7% Smix did not irritate the skin.

Based on the description above, using Smix, which is quite high in microemulsions that can increase the solubility and stability of forskolin, is expected to produce a safe microemulsion formula.

## MATERIALS AND METHODS

### Materials

Forskolin was purchased from BOCSCI (USA), Maisine<sup>®</sup> CC oil was donated from Gattefosse (France), and MCT oil was purchased from Okusi Biotech Asia (Indonesia). The surfactants used were polyoxyethylene sorbitan 20 (POE 20) purchased from Croda (Singapore), polyoxyethylene sorbitan 80 (POE 80) purchased from Seffix (Singapore), and sorbitan monolaurate (Span 20) purchased from Sigma-Aldrich (USA). The cosurfactants used were polyethylene glycol (PEG) 400, purchased from Indokemika (Indonesia), and propylene glycol (PG), purchased from Dow Chemical Pacific (Singapore). Sodium hydroxide, potassium dihydrogen phosphate, ethanol absolute, and acetonitrile were purchased from Merck (Germany), and distilled water was purchased from IPHA Lab (Indonesia).

### Methods

#### Solubility test

The solubility test was performed by gradually adding 5 mg of forskolin to 1 mL of each microemulsion-forming material (oil, surfactant, and cosurfactant) until saturated. The sample was stirred using an orbital shaker for 48 h at 25 °C at 100 rpm. Then, it was centrifuged at 6,000 rpm for 10 min and filtered through a 0.45 µm membrane (Sartorius).<sup>21</sup>

Forskolin concentration in the sample was determined by diluting the sample 20 times with ethanol. Then, it was injected into high performance liquid chromatography (HPLC) (Waters 2.487) system with column C18 (Cap Cell) and eluent water:acetonitrile (35:65).<sup>15</sup> The chromatogram was detected at a maximum  $\lambda$  of 210 nm with a retention time of 6.5 min.

#### Pseudoternary phase diagram construction

The pseudoternary phase diagrams were prepared at room temperature by mixing oil and Smix in a ratio of 1:9 to 9:1, then dripping water to form a clear microemulsion. Smix comparisons were made with surfactant: cosurfactant ratios of 1:1, 2:1, and 3:1. The pseudoternary diagram was created using the Ternary Diagram ProSim SA application version 1.0.3.0.

#### Microemulsion formulation and characterization

Microemulsions are prepared by mixing forskolin in oil and Smix. The aqueous phase was added slowly, while stirring with a magnetic stirrer at 300 rpm for 10 min at room temperature. The resulting microemulsion was characterized for its clarity by measuring light transmittance (T%) with a spectrophotometer (Beckman Coulter DU<sup>®</sup> 720 ultraviolet/visible) at a maximum  $\lambda$  of 630 nm,<sup>22,23</sup> globule size and homogeneity using a particle analyzer (Beckman Coulter DelsaTM Nano C), pH formula with a pH meter (SevenEasy Mettler Toledo), zeta potential using a zeta analyzer (Beckman Coulter DelsaTM Nano C), and phase separation test using the centrifugation method (Hettich EBA 200) at 3500 rpm for 30 minutes.<sup>24</sup>

#### Optimization of the forskolin concentration in microemulsions

Optimization of forskolin concentration in microemulsions was determined by mixing forskolin in the range of 1–5 mg.mL<sup>-1</sup>

into the formula. The dissolved forskolin concentrations were determined by HPLC.

#### Characterization of the shape and size of microemulsion globules

The shape and size of the microemulsion globules were determined by transmission electron microscopy (TEM). Microemulsion was dropped on the preparation, then covered with a 400-mesh grid, and left for approximately 1 min to absorb. Next, uranyl acetate was dropped on the grid and left for 30 min to dry and read with TEM.

#### Microemulsion spectrum characterization

The spectrum of the microemulsion formula and the interactions between forskolin and the ingredients in the microemulsion formula were analyzed by fourier transform infrared-attenuated total reflectance (FTIR-ATR) (Agilent Technologies).

#### Microemulsion stability test

The stability test for liquid preparations refers to the International Council for Harmonisation (ICH) guidelines,<sup>25</sup> where the samples were stored in the refrigerator ( $5 \pm 3^\circ\text{C}$ ), room temperature [ $30 \pm 2^\circ\text{C}$  and  $65 \pm 5\%$  relative humidity (RH)], and in the climatic chamber ( $40 \pm 2^\circ\text{C}$  and  $75 \pm 5\%$  RH) (Hotpack model 317322, USA) for accelerated stability testing. The stability of the samples was tested at storage periods of 1, 7, 14, 21, 30, 60, and 90 days by measuring the globule size, forskolin concentration, and pH of the formula.

#### Irritation test

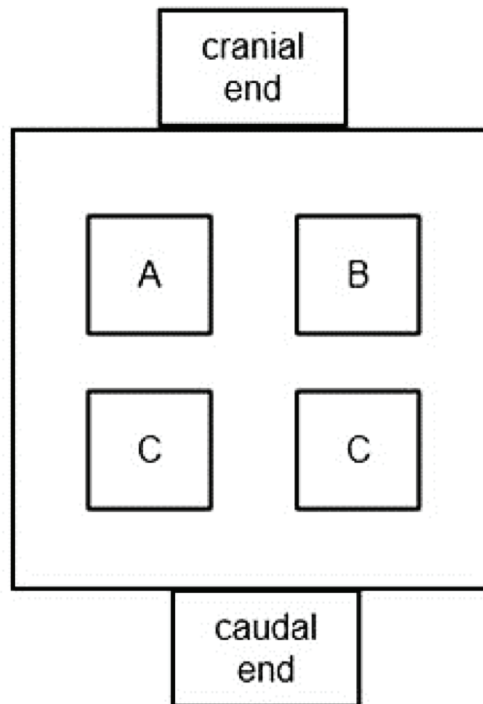
The safety of using microemulsion topically was determined through an *in vivo* skin irritation test on albino rabbits. The irritation test was approved by the Ethics Committee for the Use of Experimental Animals (KEPHP) of the Bandung Institute of Technology (no: 02/KEPHP/-ITB/10-2021.)

The irritation test refers to the guidelines for non-clinical toxicity tests *in vivo*.<sup>26</sup> The irritation test was performed on three New Zealand strain male albino rabbits. The animal's back was shaved with an area of about  $10\text{-}15\text{ cm}^2$ , then divided into four locations with a size of  $2 \times 3\text{ cm}^2$  each. At each location, 0.5 g of sample consist of microemulsion formula, microemulsion base, and 2 sites were applied as a control, which was not treated. The layout of the location of the formula application is given in Figure 2. Then, the application site is covered with gauze and covered with a bandage for 24 h, after which the gauze is removed. The appearance of erythema and edema was observed at 24, 48, and 72 h after the gauze was removed. The severity of the effect was scored between 0 and 4, with a score of 0: no erythema, 1: very little erythema, 2: well-defined erythema, 3: moderate erythema, and 4: severe erythema, as well as for edema.

The primary irritation index (PII) is calculated using the following formula:

$$\text{PII} = (\text{A}-\text{B})/\text{C}\dots\dots (\text{equation } 1)$$

where A: sum of erythema and edema scores at all locations given the test microemulsion at observations at 24, 48, and 72 h divided by the number of observations, B: sum of erythema and



**Figure 2.** Sites of formula application in irritation test

A: Microemulsion, B: Microemulsion base, C: Untreated control

edema scores for all control locations at 24, 48, and 72 h divided by the number of observations, and C: number of animals.

The level of irritation is determined on the basis of PII, that is, if PII: 0.0-0.4, the test sample is categorized as very mild irritation (negligible); 0.5-1.9 is a mild irritant (slightly); 2.0-4.9 is a moderate irritant, and 5.0-8.0 is a strong irritant (severe).

In this study, statistical data were not used.

## RESULTS AND DISCUSSION

#### Solubility test

The solubility test showed that forskolin is dissolved easily in materials containing polyoxyethylene groups, as shown in Table 1. This group is found in surfactant and cosurfactant molecules. Based on its molecular structure, forskolin has one acetate ester, a cyclic ketone, and a tertiary alpha hydroxyl group, as well as three heterocyclic rings and a hydroxyl group.<sup>27</sup> The presence of ketone and ester groups on forskolin molecules tends to form hydrogen bonds with polyoxyethylene groups on surfactant and cosurfactant molecules.

The solubility of forskolin in oil displays that forskolin is more soluble in Maisine<sup>®</sup> CC than MCT. Maisine<sup>®</sup> CC is a triglyceride oil with long-chain fatty acids derived from oleic and linoleic acids (C18:1, C18:2). Meanwhile, MCT is a triglyceride oil consisting of medium chain saturated fatty acids (C6-C12). Based on the results of this solubility test, the interaction between forskolin and oil probably occurs in the fatty acid chains of the oil and forskolin molecules through van der Waals interactions.<sup>28</sup>

The solubility test of forskolin in surfactants showed that forskolin was more soluble in POE 80 (22.97 mg/mL) than

**Table 1. Forskolin solubility in materials for microemulsion formulations**

| Materials               | Forskolin solubility, mg.mL <sup>-1</sup> ± SD |
|-------------------------|--|
| MCT                     | 3.24 ± 0.06                                    |
| Maisine <sup>®</sup> CC | 5.23 ± 0.22                                    |
| POE 20                  | 20.88 ± 0.73                                   |
| POE 80                  | 22.97 ± 0.19                                   |
| Span 20                 | 2.11 ± 0.04                                    |
| PG                      | 4.67 ± 0.10                                    |
| PEG 400                 | 23.08 ± 0.10                                   |

n: 3, SD: Standard deviation, MCT: Medium chain triglyceride, POE: Polyoxyethylene sorbitan, PG: Propylene glycol, PEG: Polyethylene glycol, Span: Sorbitan monolaurate

in POE 20 (20.88 mg/mL). However, in preliminary tests of formulation, it was found that POE 20 could mix with 4% Maisine<sup>®</sup> CC, whereas POE 80 could only mix with 2% Maisine<sup>®</sup> CC at the same surfactant concentration. This may be related to the fatty acid groups of the oil and surfactants.

The solubility test of forskolin in cosurfactant showed that forskolin was more soluble in PEG 400 (23.08 mg/mL) than in PG (4.67 mg/mL). PEG 400 consists of 8 ethylene molecules and one primary hydroxyl group, whereas PG is propane-1,2-diol with two hydroxyl groups in each molecule.<sup>29</sup> The presence of 8

ethylene molecules in PEG 400 allows it to form more hydrogen bonds to interact with forskolin molecules compared with PG. PEG 400 log P value is -4.8, whereas that of PG is -1.34.<sup>30</sup> This shows that PEG 400 is more hydrophilic than PG; therefore, it is easier to form hydrogen bonds in *o/w* microemulsions.

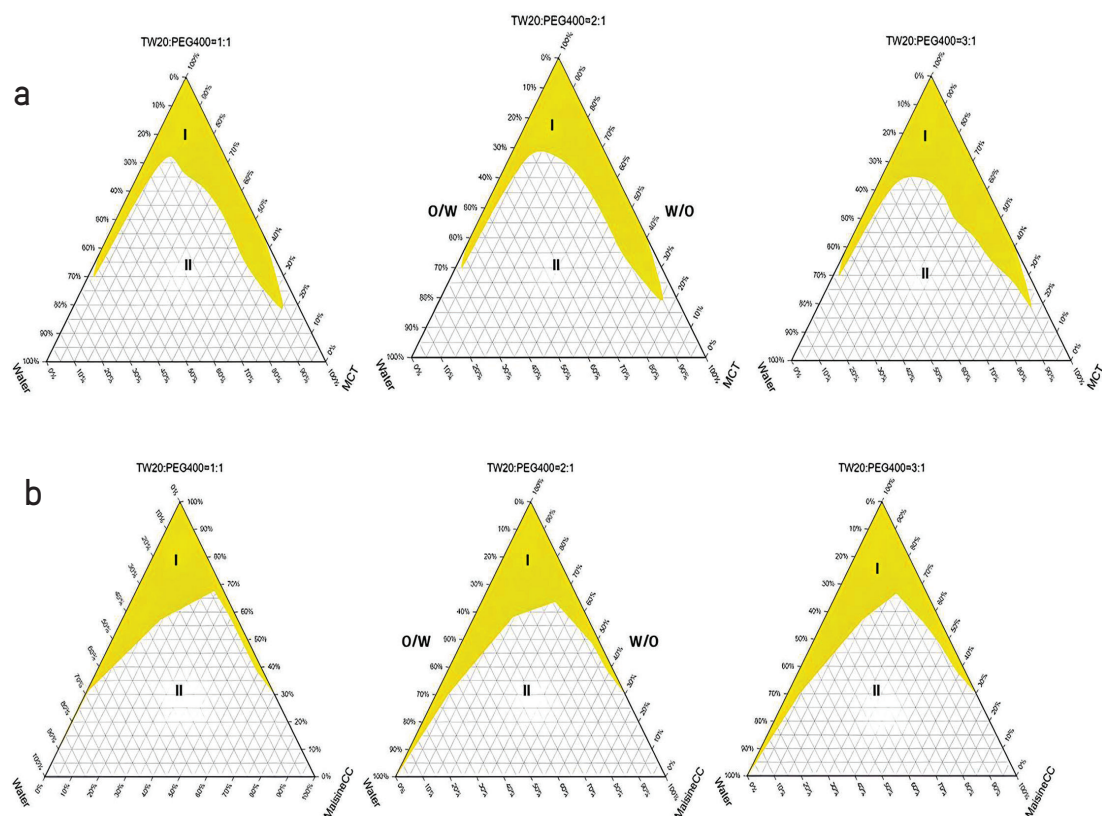
Based on the solubility test data and the preliminary formulation test, the microemulsion components used for the formulation were MCT, Maisine<sup>®</sup> CC, POE 20, and PEG 400.

#### Pseudoternary phase diagram construction

Pseudoternary phase diagrams were prepared to compare the microemulsion area formed between microemulsion-MCT (FMEA) and microemulsion-Maisine<sup>®</sup> CC (FMEB) with a ratio of Smix POE 20-PEG 400 at 1:1, 2:1, and 3:1 (*w/w*). Figure 3 illustrates that the Smix ratio shows no difference between the two types of microemulsions. However, there are differences between FMEA and FMEB. The figure shows that the interaction between MCT and Smix tends to form *w/o* microemulsions, whereas the interactions between Maisine<sup>®</sup> CC and Smix tend to form *o/w* microemulsions.

#### Microemulsion formulation

Microemulsions are colloidal dispersions similar to other drug delivery systems, which contain high concentrations of surfactants. Table 2a shows that FMEA1 was formed by MCT:POE 20:PEG 400 (2:27.5:10 *w/v*), whereas the FMEB1 formula was formed by Maisine<sup>®</sup> CC:POE 20:PEG 400 (4:25:5 *w/v*). Increasing the amount of oil in each formula increases



**Figure 3.** Microemulsion pseudoternary phase diagrams. (a) Pseudoternary phase diagrams with MCT oil, (b) pseudoternary phase diagrams with Maisine<sup>®</sup>, I: microemulsion area, II: emulsion area

turbidity and globule size and decreases the percentage transmission (T%) value.

In the FMEA1 formula, the interaction between POE 20 and MCT is unstable, which is presumably because the lipophilic part of POE 20 is difficult to interact with MCT, which is oil from saturated fatty acids (C6-C12). The interaction between POE 20 and Maisine® CC on FMEB1 shows that the system can form stable microemulsions. POE 20 tends to interact more easily with Maisine® CC oil, which has unsaturated fatty acid groups from oleic and linoleic acids, which can form *o/w* microemulsions. Hathout et al.<sup>9</sup> found that POE 20 can interact with oleic acid to form an *o/w* system in a testosterone microemulsion. Oleic acid and Maisine® CC are both unsaturated fatty acid (C18) oils. In Table 2b, FMEB1, which comprises Maisine® CC:POE 20:PEG 400 at 4:25:5 w/v, is the optimum formula. This formula gives a T% value of 100.67%, which shows that the formula can transmit all light, giving a clear appearance. Other formulas provide a T% value of less than 90%. T% value can be used as a reference when selecting the microemulsion formula. The level of clarity of the formula is also related to the size of the globules. The FMEB1 formula has a globule size of approximately 28 nm and homogeneity value of approximately 0.3.

The zeta potential values of all formulations range from -1.40 mV to -1.74 mV, indicating that the formulas are non-ionic. The

centrifugation test showed that only the FMEB1 formula was homogeneous, while the others were separated. Based on the test results, FMEB1 can be used in the next stage of testing.

*Optimization of forskolin concentrations in microemulsions*

Optimization of forskolin solubility in the FMEB1 formula is shown in Table 3, where FMEB1 can dissolve forskolin by 2.19 ± 0.05 mg/mL. The addition of forskolin concentration starting at 3 mg mL<sup>-1</sup> gave the appearance of cloudy microemulsions, large globule size and precipitate. This indicates that FMEB1 has been saturated; therefore, the next concentration of forskolin used is 2 mg.mL<sup>-1</sup> or 0.2%.

*Characterization of the shape and size of microemulsion globules*

TEM results (Figure 4) indicated globule-shaped droplets of FMEB1 with a size of approximately 30-50 nm.

**Table 2a. Composition of microemulsion formulations**

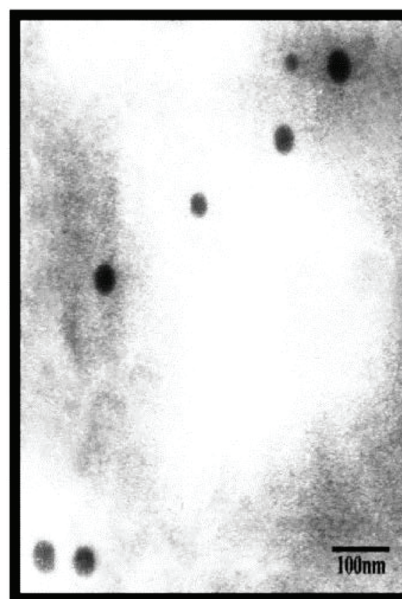
| Materials, w/v % | Formulations |       |       |       |
|------------------|--------------|-------|-------|-------|
|                  | FMEA1        | FMEA2 | FMEB1 | FMEB2 |
| Forskolin        | 0.2          | 0.2   | 0.2   | 0.2   |
| MCT              | 2            | 3     | -     | -     |
| Maisine® CC      | -            | -     | 4     | 5     |
| POE 20           | 27.5         | 27.5  | 25    | 25    |
| PEG 400          | 10           | 10    | 5     | 5     |
| Water            | 60.5         | 59.5  | 66    | 65    |

FMEA: Forskolin microemulsion with MCT oil, FMEB: Forskolin microemulsion with Maisine® CC oil, w/v: Weight/volume

**Table 2b. Characteristics of microemulsion formulations**

| Test                    | Formulations |        |               |              |
|-------------------------|--------------|--------|---------------|--------------|
|                         | FMEA1        | FMEA2  | FMEB1         | FMEB2        |
| Appearance              | Bluish       | Cloudy | Transparent   | Bluish       |
| T, % ± SD               | 63.9 ± 0.15  | nd     | 100.67 ± 0.26 | 85.27 ± 0.66 |
| Globules size, nm ± SD  | 76.67 ± 5.82 | nd     | 28.80 ± 2.85  | 74.11 ± 6.00 |
| PI ± SD                 | 0.30 ± 0.05  | nd     | 0.31 ± 0.17   | 0.40 ± 0.02  |
| pH ± SD                 | 6.34 ± 0.02  | nd     | 6.23 ± 0.06   | 6.25 ± 0.01  |
| Zeta potential, mV ± SD | -1.40 ± 1.22 | nd     | -1.73 ± 1.87  | -1.74 ± 0.76 |
| Centrifuge              | Separation   | nd     | Homogenous    | Separation   |

FMEA: Forskolin microemulsion with MCT oil, FMEB: Forskolin microemulsion with Maisine® CC oil, T: Transmittance, PI: Polydispersity index, nd: No detected, SD: Standard deviation



**Figure 4.** TEM results from FMEB1 at 40.000x magnification  
TEM: Transmission electron microscopy, FMEB: Optimum microemulsion formula

### Microemulsion spectrum characterization

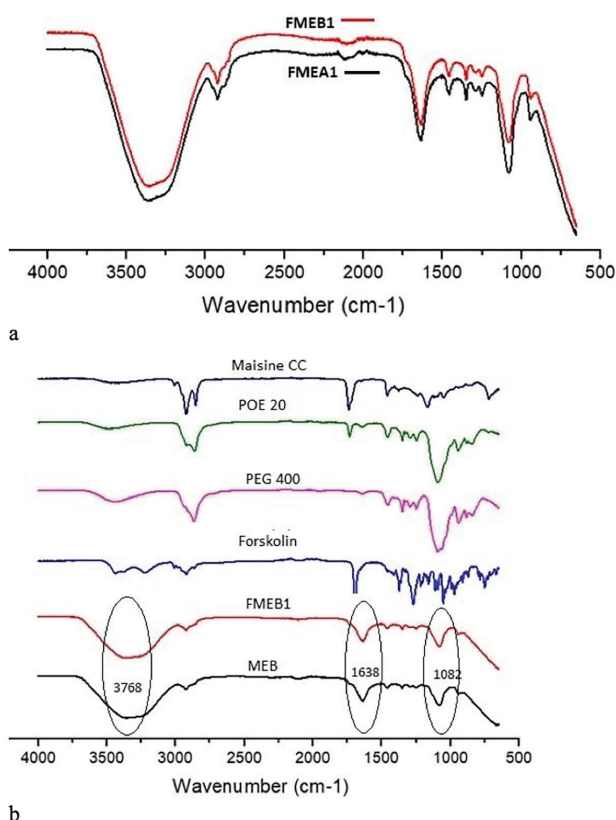
The FTIR spectra of FMEA1 and FMEB1 microemulsions are shown in Figure 5a, and the spectra of the microemulsion ingredients and forskolin interactions in FMEB1 are shown in Figure 5b.

As shown in Figure 5a, there is no significant difference between the FMEA1 and FMEB1 spectra. These data indicate the length of the fatty acid chains in the triglyceride oils used in FMEA1 and FMEB1 does not show any difference in FTIR spectrum.

Figure 5b shows the same spectrum of FMEB1 and MEB at 3,768  $\text{cm}^{-1}$ : OH stretching of a water molecule; 1,638  $\text{cm}^{-1}$ : stretching of the C=O functional group due to the conjugation effect of the aldehyde group on Maisine<sup>®</sup> CC, POE 20, and forskolin; 1,082  $\text{cm}^{-1}$ : stretching of the C-O group due to the interaction between POE 20 and PEG 400.<sup>31</sup> From the spectrum of that figure, there are no new bands indicating a strong interaction between molecules or it can be concluded that the interactions that occur in the microemulsion system are weak interactions such as van der Waals forces or hydrogen bonds.

### Microemulsion stability test

The size of FMEB1 globules is shown in Table 3. The sizes of the FMEB1 globules stored in the refrigerator (16-17 nm) and



**Figure 5.** (a) Comparison of the FTIR spectrum pattern between FMEA1 and FMEB1 formula. (b) FTIR spectrum of forskolin and microemulsion constituent in FMEB1

FMEA1: Microemulsion with MCT oil, FMEB1: Microemulsion with Maisine<sup>®</sup> CC oil, FTIR: Fourier transform infrared, POE: Polyoxyethylene sorbitan, PEG: Polyethylene glycol, MEB: Microemulsion base

RT (17-27 nm) are still within the microemulsion requirements of < 50 nm.<sup>32</sup> In contrast, in the accelerated test, there was an increase in globule size from the 21<sup>st</sup> day at the end of the test with (115-942 nm).

The instability of the globule size in the accelerated test was probably due to the increase in temperature, which caused stretching of the polyoxyethylene groups of POE 20, resulting in reduced hydrogen bonding between the ethylene groups of the surfactants and water. This condition causes a decrease in the hydrophilic nature of POE 20, so it cannot dissolve in water.<sup>33</sup> This phenomenon causes POE 20 to be unable to reduce the surface tension, which causes the microemulsion monolayer membrane to break, resulting in coalescence and phase separation in the microemulsion.

The concentration of forskolin in FMEB1 determined in the stability test can be seen in Figure 6 and pH in Figure 7.

Weng and Huang<sup>16</sup> investigated the kinetics of forskolin degradation in an aqueous environment and found that the stability of forskolin was affected by pH and storage temperature. Forskolin is relatively stable at pH 3.5-5 and temperature < 50 °C. At pH > 6.5 and temperature > 50 °C, forskolin tends to hydrolyze to 7-diacetyl forskolin (forskolin-D), which then undergoes isomerization to isoforskolin. Yamamura et al.<sup>34</sup> developed a forskolin emulsion with a globule size of 204 nm, which was stable for 30 days, and found that forskolin was incorporated into the monolayer membrane to protect it from hydrolysis. The finding of stable levels of forskolin in FMEB1 indicates that forskolin is thought to be incorporated into the monolayer membrane formed by Smix used in this study.

### Irritation test

The results of the irritation test are shown in Table 5 and Figure 8.

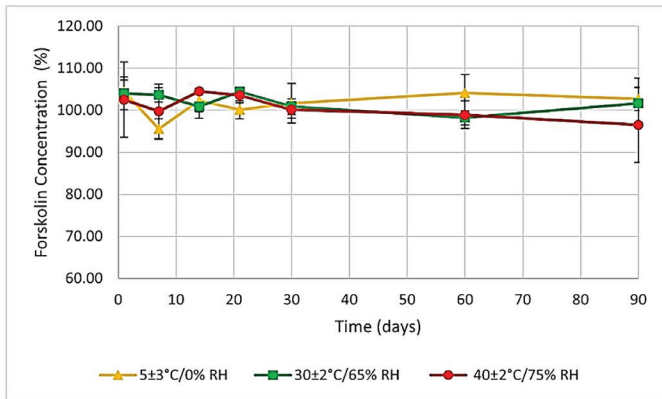
In Table 5, PII FMEB1 is classified as a very weak and negligible irritant (PII: 0.11), where erythema only occurs in 1 rabbit at T24 with a score: 1, then disappears. These data indicate the use of 25% POE 20 and 5% PEG 400 as Smix, both of which are composed of non-irritating polyoxyethylene groups. The results of this test agree with Rhein et al.<sup>35</sup> Rhein found that the use of 1-30% w/w non-ionic surfactants or cosurfactants that had polyoxyethylene groups did not cause swelling.

Swelling is a condition in which the *stratum corneum* is hydrated, which can absorb 5 to 6 times its weight in water<sup>36</sup> and can cause edema as one of the effects of irritation. Swelling occurs due to ionic interactions between water molecules in the skin and surfactant hydrophilic groups and the interaction of surfactant alkyl chains with corneocytes. The type of hydrophilic groups in the surfactants is a determining factor for swelling. POE 20 used in FMEB1 is non-irritating. This is due to the interaction between the polyoxyethylene groups in POE 20 and water molecules in the skin only forming weak interactions in the form of hydrogen bonds so that these interactions do not cause swelling.<sup>35,37</sup> PEG 400 is a hydrophilic molecule with a distribution coefficient of 0.000015<sup>30</sup>. This makes it difficult for this molecule to interact with the *stratum corneum* and only acts

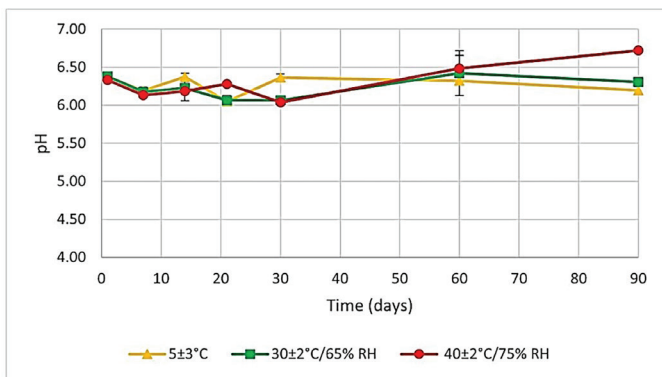
**Table 3. Optimization of forskolin concentration in FMEB1 formula and globule size**

| Forskolin concentration in FMEB1, mg/mL | Forskolin solubility, mg/mL ± SD | Globule size, nm ± SD | PI ± SD     | Appearance           |
|---|----------------------------------|-----------------------|-------------|----------------------|
| 1                                       | 0.98 ± 0.01                      | 26.06 ± 3.44          | 0.27 ± 0.01 | Transparent          |
| 2                                       | 1.89 ± 0.02                      | 24.03 ± 4.11          | 0.15 ± 0.03 | Transparent          |
| 3                                       | 2.25 ± 0.03                      | 19.63 ± 0.67          | 0.28 ± 0.09 | Bluish (precipitate) |
| 4                                       | 2.18 ± 0.03                      | 3220.37 ± 492.85      | 0.76 ± 0.18 | Cloudy               |
| 5                                       | 2.14 ± 0.03                      | 3147.80 ± 408.98      | 0.81 ± 0.12 | Cloudy               |
| Å                                       | 2.19 ± 0.05                      | -                     | -           | -                    |

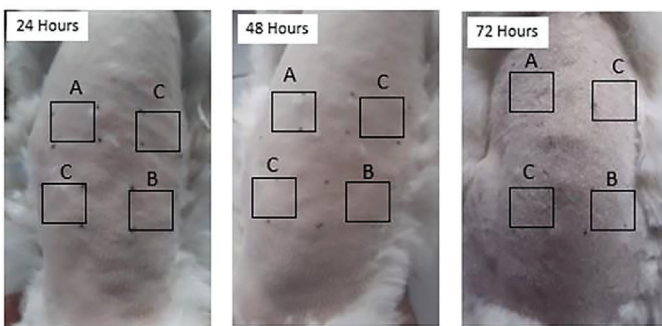
n: 3, Å: Mean of FSK solubility in FMEB1, SD: Standard deviation, PI: Primary irritation, FMEB: Forskolin microemulsion with Maisine® CC oil



**Figure 6.** Stability study of forskolin concentration test in FMEB1, n: 3



**Figure 7.** Stability study of pH in FMEB1, n: 3



**Figure 8.** Results of irritation test on the skin of albino rabbits, A: FMEB1, B: MEB, and C: untreated control  
FMEB1: Microemulsion with Maisine® CC oil, MEB: Microemulsion base

**Table 4. Stability study of globule size in FMEB1**

| Time (days) | Globule size (nm) at storage condition |              |                |
|-------------|--|--------------|----------------|
|             | 5 °C/0% RH                             | 30 °C/65% RH | 40 °C/75% RH   |
| 1           | 16.70 ± 1.59                           | 21.73 ± 6.05 | 21.63 ± 4.09   |
| 7           | 16.63 ± 1.10                           | 24.28 ± 6.70 | 21.74 ± 2.44   |
| 14          | 17.13 ± 2.93                           | 19.73 ± 2.21 | 21.94 ± 1.05   |
| 21          | 17.00 ± 1.32                           | 21.88 ± 2.33 | 115.37 ± 11.79 |
| 30          | 17.82 ± 0.52                           | 21.40 ± 2.96 | 108.6 ± 8.00   |
| 60          | 17.30 ± 2.92                           | 17.9 ± 2.36  | 409.10 ± 68.03 |
| 90          | 17.53 ± 1.65                           | 27.00 ± 2.46 | 942.63 ± 28.92 |

n: 3, RH: Relative humidity

**Table 5. Skin irritation test result**

| Group             | Time (hours) | Total score |   | PII  |
|-------------------|--------------|-------------|---|------|
|                   |              | E           | O |      |
| FMEB1             | 24           | 1           | 0 | 0.11 |
|                   | 48           | 0           | 0 |      |
|                   | 72           | 0           | 0 |      |
| MEB               | 24           | 0           | 0 | 0    |
|                   | 48           | 0           | 0 |      |
|                   | 72           | 0           | 0 |      |
| Untreated control | 24           | 0           | 0 | -    |
|                   | 48           | 0           | 0 |      |
|                   | 72           | 0           | 0 |      |

n: 3 (number of rabbits), E: Erythema, O: Oedema, PII: Primary irritation index, FMEB1: Optimum microemulsion formula, MEB: Microemulsion base

as a carrier in the delivery system.<sup>38</sup> Therefore, the POE 20 and PEG 400 used in this study are not irritants.

**CONCLUSION**

Forskolin can be expressed as a microemulsion using the phase titration method. The optimum formula consisted of Maisine® CC, POE 20, and PEG 400 (4:25:5 w/v) with a forskolin

concentration of 0.2%. Microemulsions are stable when stored in the refrigerator and at room temperature, and are very mildly irritating and can be ignored with PII: 0.11.

With the successful preparation of stable forskolin microemulsion, there is an opportunity to use forskolin topically as a candidate for lipolysis. For further research, *in vitro* diffusion tests, lipolysis effects, histopathology, and cytotoxicity tests will be performed to ensure the efficacy and safety of the formula.

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### Ethics

**Ethics Committee Approval:** This research received ethical approval number: 02/KEPHP/ITB/10-2021 from the Ethical Commission for the Use of Experimental Animals, School of Pharmacy, Institute of Technology Bandung.

**Informed Consent:** Not applicable.

**Peer-review:** Externally peer-reviewed.

### Authorship Contributions

Concept: R.N., Y.C.S., S.T.D., M.I.I., Design: Y.C.S., S.T.D., M.I.I., Data Collection or Processing: R.N., S.T.D., Analysis or Interpretation: R.N., Y.C.S., S.T.D., Literature Search: R.N., M.I.I., Writing: R.N., S.T.D., M.I.I.

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