

Development of Forskolin Microemulsion Formula and its Irritation Test on Rabbits

Short Title: Forskolin Microemulsion Formula and Irritation Test

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ABSTRACT

Objective: This study aims to develop a microemulsion formula that can increase the solubility and stability of forskolin and is safe for topical use.

Materials and Methods: The materials used for the development of the microemulsion formula were triglyceride oil, nonionic surfactants, and polyethylene glycol for cosurfactants which were selected based on the results of the forskolin solubility test using high performance liquid chromatography. Microemulsion was formulated by phase titration method. Formula stability was determined on storage for 90 days at refrigerator, room temperature, and accelerated stability test by determining globule size, forskolin concentration, and pH. The safety of using microemulsion was determined by skin irritation tests on albino rabbits.

Results: The optimum microemulsion formula consisted of Maisine CC, polyoxyethylene sorbitan 20, and polyethylene glycol 400 with a ratio of 4:25:5 w/v which increased the solubility of forskolin the most, namely 2.19 mg.mL⁻¹. Based on globule size (<50nm), forskolin concentration (2 mg.mL⁻¹), 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 shows a primary irritation index of 0.11 which is categorized as very weak irritation and can be ignored.

Conclusion: The microemulsion prepared by the phase titration method containing Maisine CC, polyoxyethylene sorbitan 20 and polyethylene glycol 400 (4:25:5, w/v) as a base and 0.2% forskolin, is stable in refrigerator storage and room temperature. This microemulsion is very mild or negligible irritant with a primary irritation index = 0.11.

Keywords: microemulsion, forskolin, skin irritation test.

INTRODUCTION

Microemulsion is a colloidal dispersion composed of water, oil, and a mixture of cosurfactants (Smix) that forms spontaneously¹. Microemulsion is a delivery system that can function as a solubility enhancer^{2,3}, penetration^{4,7}, stability, diffusion, and is able to dissolve lipophilic and hydrophilic Active Pharmaceutical Ingredients (API)⁸. 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 corneum⁹, while surfactants can reduce surface tension, increase API solubility, and increase skin penetration¹⁰. The cosurfactant functions to increase 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 root of *Coleus forskohlii*. This plant is often found in India, Nepal, Burma, and Thailand¹¹. One of the pharmacological effects of forskolin is as a lipolytic agent, where forskolin can activate the adenylate cyclase (AC) enzyme in adipose cells to stimulate the formation of cyclic adenosine mono phosphate (cAMP). In vitro, forskolin can induce lipolysis by increasing the concentration of cAMP and the formation

of protein kinase A (PKA)¹²⁻¹⁴. Organoleptically, forskolin is in the form of a white powder with a molecular weight of 410.5 grams.mole⁻¹¹⁵. The molecular structure of forskolin can be seen in Figure 1. Forskolin is insoluble in water with log P = 3.89¹⁵ but undergoes hydrolysis and isomerization at temperatures above 50°C¹⁶.

Forskolin is generally formulated in capsule dosage forms and is used orally as a slimming agent. The development of formulas in topical dosage forms can increase their usefulness. Microemulsion was chosen as a delivery system for forskolin because this system can increase solubility through the interaction of 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. It is known that a high monoglyceride content in oil can increase the solubility of API with log P > 2¹⁷. Acharya et al.² found that a microemulsion containing triglyceride oil can increase the solubility of aceclofenac. In this study, a microemulsion was formulated based on the length of the carbon chain of triglyceride oil. The oil used is Maisine CC which is long chain triglyceride oil of oleic and linoleic unsaturated fatty acids (C18:1, C18:2) and MCT which is medium chain triglyceride oil of caproic, caprylic, capric and lauric saturated fatty acids (C6 – C12)¹⁸.

One of the undesirable effects of microemulsions on the skin is their irritating properties due to high concentrations of surfactants. Mu et al. found that a rotigotine microemulsion containing 25.2% Smix did not irritate the skin¹⁹. Soliman et al also found that the celecoxib microemulsion containing 45.7% Smix did not cause irritation to the skin²⁰.

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

MATERIALS AND METHODS

Materials

Forskolin was purchased from BOCSCI (USA), Maisine[®] CC oil was donated from Gattefosse (France), Middle Chain Triglyceride (MCT) oil was purchased from Okusi Biotech Asia (Indonesia). The surfactants used were solyoxyethylene 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). While the cosurfactants used are 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 carried out 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 hours at 25°C with a speed of 100 rpm. Then it was centrifuged at 6000 rpm for 10 minutes and filtered through a 0.45µm membrane (Sartorius)²¹.

Determination of forskolin concentration in the sample was determined by diluting the sample 20 times with ethanol. Then it was injected into column C18 (Cap Cell) High Performance Liquid Chromatography (HPLC) (Waters 2487) with the eluent water:acetonitrile (35:65)¹⁵. The chromatogram was detected at a maximum λ of 210 nm with a retention time of 6.5 minutes.

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 made by mixing forskolin in oil and Smix. The aqueous phase was added slowly while stirring with a magnetic stirrer at 300 rpm for 10 minutes at room temperature. The resulting microemulsion was characterized for its clarity by measuring light transmittance (T%) with a spectrophotometer (Beckman Coulter DU[®] 720 UV/Vis) at a maximum λ of 630nm^{22,23}, 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 minutes²⁴.

Optimization of forskolin concentration in microemulsions

Optimization of forskolin concentration in microemulsions was determined by mixing forskolin in the range of 1-5 mg.mL⁻¹ into the formula. Dissolved forskolin concentrations were determined by HPLC.

Characterization of 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 400-mesh grid and left for approximately one minute to absorb. Next, drop uranyl acetate on the grid and left it for 30 minutes 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 analysed by Fourier Transform Infra Red – Attenuated Total Reflectance (FTIR-ATR) (Agilent Technologies).

Microemulsion Stability Test

The stability test for liquid preparations refers to the ICH guidelines²⁵, where the samples are 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 test. The stability of the samples was tested at storage periods of 1, 7, 14, 21, 30, 60 and 90 days by measuring 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 with no. 02/KEPHP-ITB/10-2021.

The irritation test refers to the guidelines for non-clinical toxicity tests in vivo²⁶. The irritation test was carried out on three New Zealand strain male albino rabbits. The animal's back was shaved with an area of about $10 \times 15 \text{ cm}^2$, then divided into four locations with a size of $2 \times 3 \text{ cm}^2$ each. At each location, 0.5 gram of sample consisting 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 can be seen in Figure 2. Then the application site is covered with gauze and covered with a bandage for 24 hours, then the gauze is removed. The appearance of erythema and oedema was observed at 24, 48, and 72 hours after the gauze was removed. The severity of the effect was scored between 0 – 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 oedema.

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

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

where A = sum of erythema and oedema scores at all locations given the test microemulsion at observations at 24, 48 and 72 hours divided by the number of observations, B = sum of erythema and oedema scores for all control locations at 24, 48 and 72 hours divided by the number of observations and C = number of animals.

The level of irritation is determined based on PII, that is, if $\text{PII} = 0.0 - 0.4$, 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).

Statistical analysis

In this study, statistical data was not used.

RESULTS AND DISCUSSION

Solubility Test

The solubility test showed that forskolin dissolves 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 group²⁷. The presence of ketone and ester groups on forskolin molecules tends to form hydrogen bonds with polyoxyethylene groups on surfactant and cosurfactant molecules.

As for the solubility of forskolin in oil, it shows that forskolin is more soluble in Maisine CC than MCT. Maisine 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 that occurs between forskolin and oil probably occurs in the fatty acid chains of the oil and forskolin molecules through Van der Waals interactions²⁸.

The solubility test of forskolin in surfactants showed that forskolin was more soluble in POE 80 (22.97 mg.mL^{-1}) than POE 20 (20.88 mg.mL^{-1}). However, in preliminary tests of formulation it was found that POE 20 could mix with 4% Maisine CC, whereas POE 80 could only mix with 2% Maisine 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^{-1}) than in propylene glycol (4.67 mg.mL^{-1}). 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 molecule²⁹. The presence of 8 ethylene molecules in PEG 400 allows it to form more hydrogen bonds to interact with forskolin molecules compared to PG. PEG 400 log P value is -4.8 , while PG is -1.34 ³⁰. This shows that PEG 400 is more hydrophilic than PG so that it will be 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 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 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 in 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, while the interactions between Maisine CC and Smix tend to form o/w microemulsions.

Microemulsion Formulation

Microemulsions are colloidal dispersions like 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) while the FMEB1 formula was formed by Maisine CC:POE 20:PEG 400 (4:25:5 w/v). Increasing the amount of oil in each formula increases turbidity and globule size and decreases the percentage transmission (T%) value.

In the FMEA1 formula, the interaction between POE 20 and MCT is unstable, this is presumably because the lipophilic part of POE 20 is difficult to interact with MCT which is oil from saturated fatty acids (C6 -C12). Whereas 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. found that POE 20 can interact with oleic acid to form an o/w system in a testosterone microemulsion⁹. It is known that oleic acid and Maisine CC are both unsaturated fatty acid (C18) oils.

In Table 2b it can be seen that FMEB1 which is composed of 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%, this shows that the formula can transmit all light so that it gives a clear appearance. The other formulas provide T% value of less than 90%. The T% value can be used as a reference in selecting 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 around 28 nm and homogeneity value of around 0.3.

The zeta potential values of all formulations range from -1.40 mV to -1.74 mV, this indicates that the formulas are nonionic. As for the centrifugation test, it showed that only the FMEB1 formula was homogeneous, while the others were separation. 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 can be seen in Table 3, where FMEB1 can dissolve forskolin by 2.19 ± 0.05 mg.mL⁻¹. The addition of forskolin concentration starting at 3 mg.mL⁻¹ gave the appearance of cloudy microemulsions, large globule size and precipitate. This indicates that FMEB1 has been saturated, so the next concentration of forskolin used is 2 mg.mL⁻¹ or 0.2%.

Characterization of Shape and Size of Microemulsion Globules

The TEM results (Figure 4) showed globule-shaped droplets of FMEB1 with a size of about 30-50 nm.

Microemulsion Spectrum Characterization

The FTIR spectrum of the FMEA1 and FMEB1 microemulsions can be seen in Figure 5a and the spectrum of the microemulsion ingredients and forskolin interactions in FMEB1 is shown in Figure 5b.

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

Figure 5b shows the same spectrum of FMEB1 and MEB at 3768cm⁻¹: OH stretching of a water molecule; 1638 cm⁻¹: stretching of the C=O functional group due to the conjugation effect of the aldehyde group on Maisine CC, POE 20 and forskolin; 1082 cm⁻¹: stretching of the C-O group due to the interaction between POE 20 and PEG 400³¹. 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 interactions weak ones such as Van der Waals forces or hydrogen bonds.

Microemulsion Stability Test

The size of the FMEB1 globules can be seen in Table 3. The sizes of the FMEB1 globules stored in the refrigerator (16-17 nm) and RT (17-27 nm) are still within the microemulsion requirements of <50 nm³². Whereas in the accelerated test there was an increase in globule size from the 21st day to the end of the test with (115 - 942 nm).

The instability of 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 surfactants and water. This condition causes a decrease in the hydrophilic nature of POE 20 so that it cannot dissolve in water³³. 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 et al. 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 to 5 and temperature < 50°C. At pH > 6.5 and temperature > 50°C, forskolin tends to hydrolyze to 7-diacetyl forskolin (Forskolin-D), then undergoes isomerization to isoforskolin¹⁶. Yamamura et al. 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 so that it was protected from hydrolysis³⁴. 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 can be seen in Table 5 and Figure 8.

In Table 5, the PII FMEB1 is classified as 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 a Smix both of which are composed of non-irritating polyoxyethylene groups. The results of this test are in line with the research by Rhein et al. Rhein found that the use of 1 - 30% w/w nonionic surfactants or cosurfactants which have polyoxyethylene groups does not cause swelling³⁵.

Swelling is a condition in which the stratum corneum is hydrated, which can absorb 5 to 6 times its weight in water³⁶ 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 surfactants is a determining factor for swelling. The 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^{35,37}. PEG 400 is a hydrophilic molecule with a distribution coefficient of 0.000015³⁰, making it difficult for this molecule to interact with the stratum corneum and only acts as a carrier in the delivery system³⁸. Therefore the POE 20 and PEG 400 used in this study are not irritants.

CONCLUSION

Forskolin can be formulated in microemulsion by phase titration method. The optimum formula consists of Maisine CC, POE 20, and PEG 400 (4:25:5 w/v) with forskolin concentration of 0.2%. Microemulsion is stable when stored in the refrigerator and at room temperature and is very mildly irritating and can be ignored with PII = 0.11.

With the successful preparation of stable forskolin microemulation, 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 carried out 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 released by Ethical Commission for the Use of Experimental Animals, School of Pharmacy, Institute of Technology Bandung.

Informed Consent: Not applicable

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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Title Manuscript: Formulation, Stability, and Irritation Test for Forskolin Microemulsion

Table 1: Forskolin solubility in materials for microemulsion formulations

Materials	Forskolin Solubility, mg.mL ⁻¹ ±SD
MCT	3.24±0.06
Maisine 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

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, n= 3

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

Forskolin Concentration in FMEB1, mg.mL-1	Forskolin Solubility, mg.mL-1 ±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
\bar{A}	2.19±0.05	-	-	-

\bar{A} = mean of FSK Solubility in FMEB1, n = 3

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

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	

E= Erythema, O= Oedema, PII= Primary Irritation Index
FMEB1 = optimum microemulsion formula, MEB = microemulsion base, n= 3 (number of rabbits)

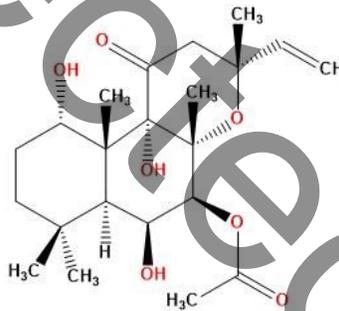


Figure 1. Molecular structure of forskolin.

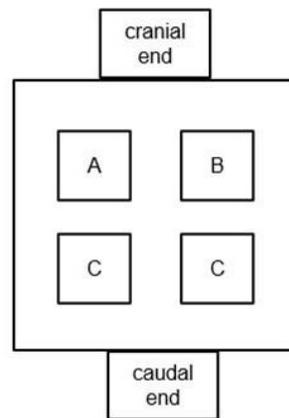


Figure 2. Sites of formula application in irritation test. A= microemulsion, B=microemulsion base, C= untreated control

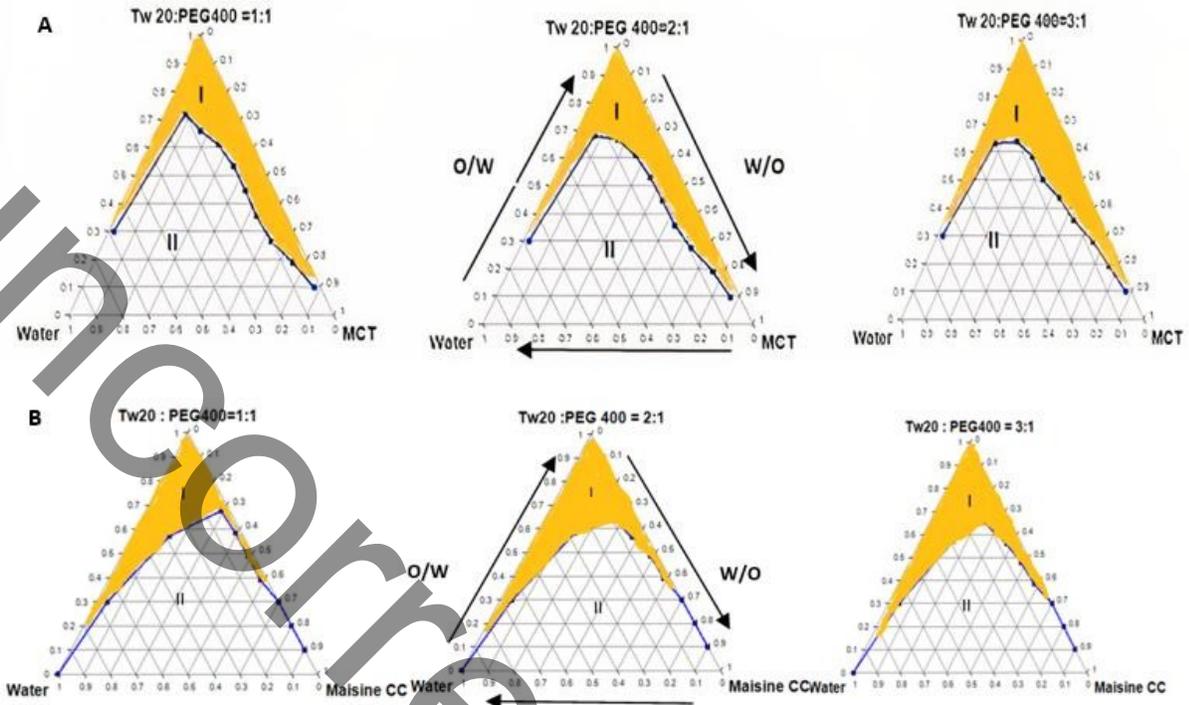


Figure 3. Microemulsion pseudoternary phase diagrams. A = pseudoternary phase diagrams with MCT oil, B = pseudoternary phase diagrams with Maisine. I = microemulsion area, II = emulsion area

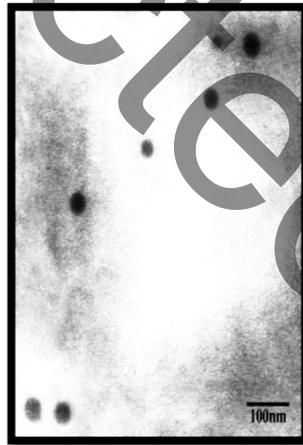


Figure 4. TEM results from FMEB1 at 40 000X magnification.

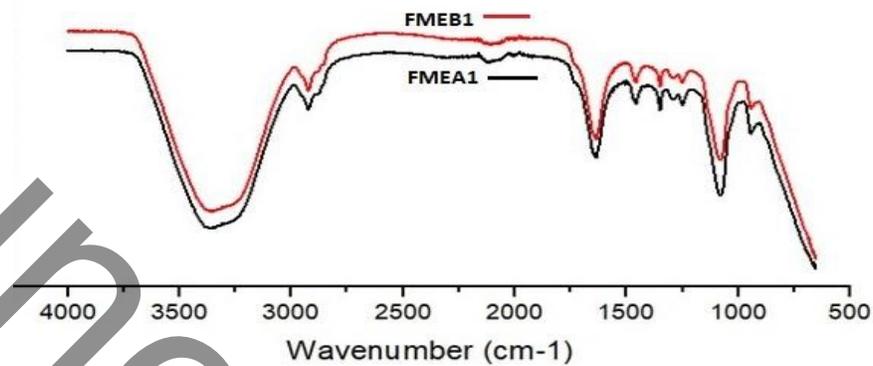


Figure 5a. Comparison of the FTIR spectrum pattern between FMEA1 and FMEB1 formula. FMEA1 = microemulsion with MCT oil, FMEB1 = microemulsion with MaisineCC oil.

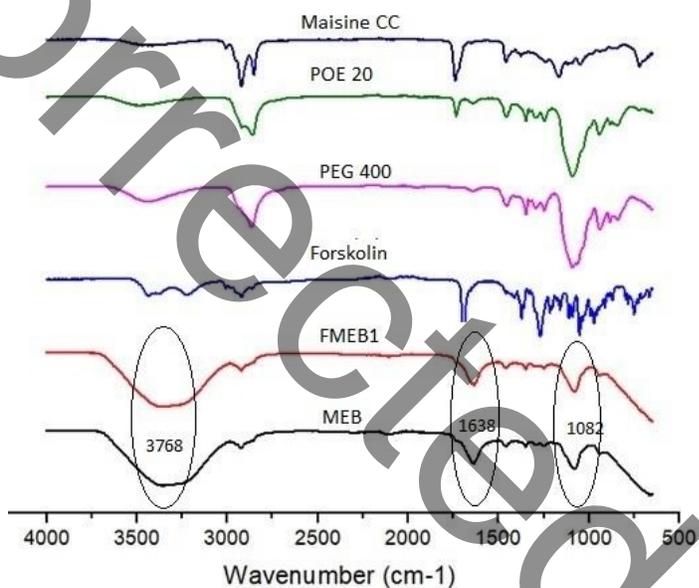


Figure 5b. FTIR spectrum of forskolin and microemulsion constituent in FMEB1.

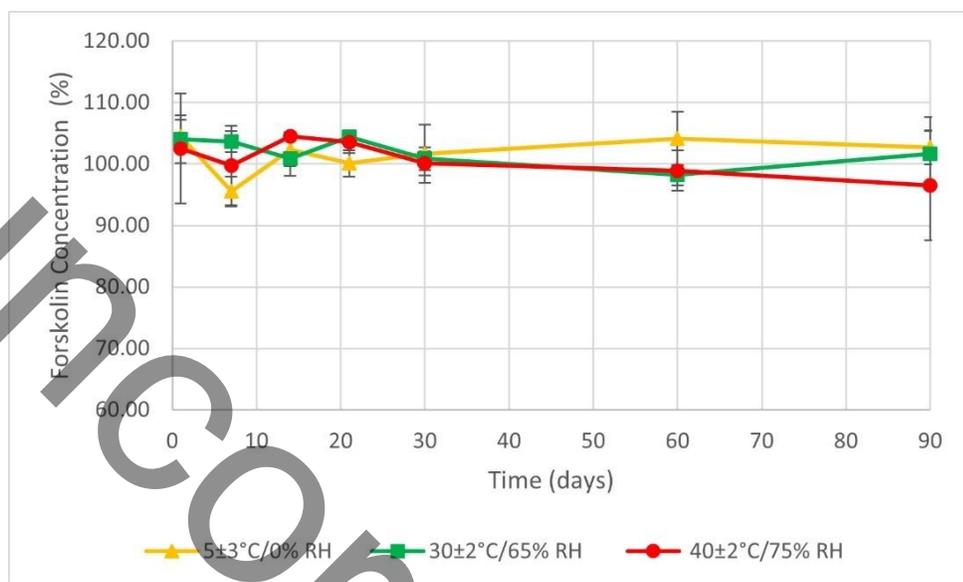


Figure 6. Stability study of forskolin concentration test in FMEB1, n=3

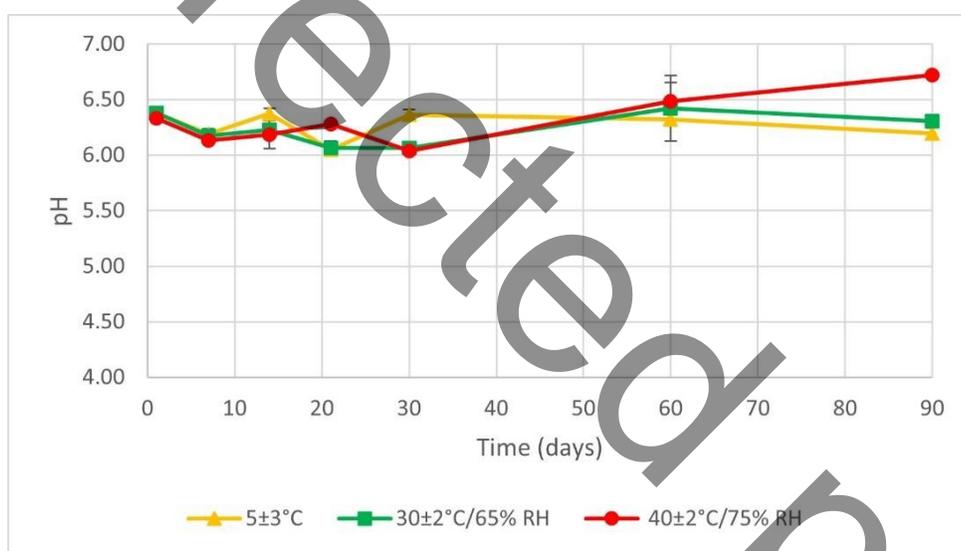


Figure 7. Stability study of pH in FMEB1, n=3

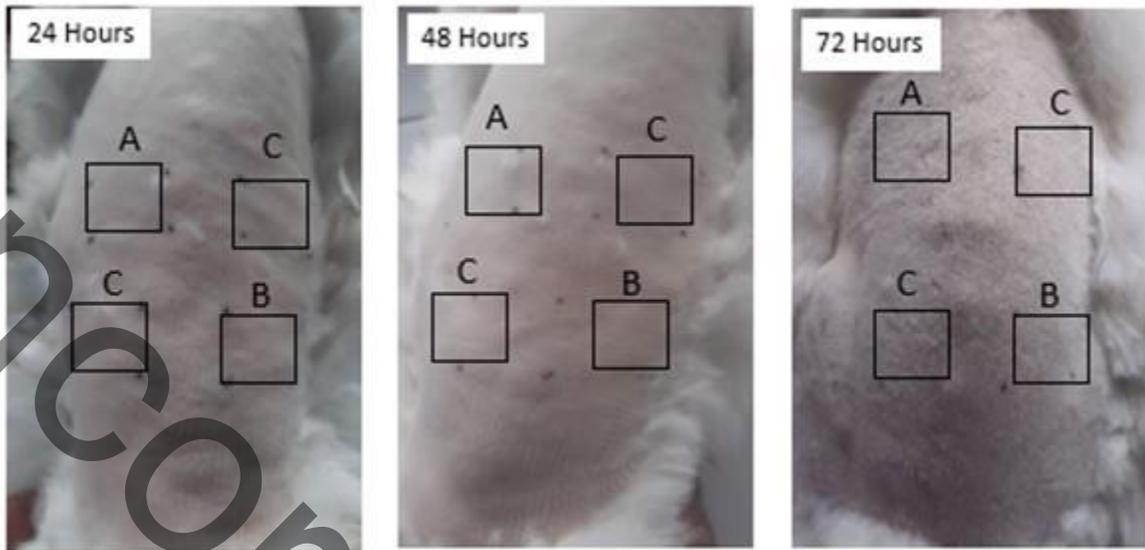


Figure 8. Results of irritation test on the skin of albino rabbits, A = FMEB1, B = MEB and C= Untreated control