

Preliminary Study of Sunscreen and Anti-Tyrosinase Effect on Microemulsion Extract from *Melaleuca Cajuputi* Essential Oil using Nonionic Surfactant

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Abstract— Global warming nowadays has led the cosmetic industry resorts to development in the field of microtechnologies. Due to this concern, the most potential sunscreen effect on microemulsion extract from *Melaleuca cajuputi* essential oil stabilized using different nonionic surfactant TWEEN 20, TWEEN 80, SPAN 20 and SPAN 80 have been investigated. All microemulsions were formulated via triangle phase diagram, emulsification process, stability test, Scanning Electron Microscope (SEM) test and Ultraviolet-Visible (UV-Vis) spectroscopy test. For triangle phase diagram, the most stable formulation microemulsions were composed of nonionic surfactant TWEEN 20, TWEEN 80, SPAN 20 and SPAN 80, olive oil and water with region have been constructed using microscopic test. For emulsification process, the compatible formulation microemulsion was taken from all formulations involved the stability test. For SEM test, the size for each droplets of microemulsion for Formulation 1, Formulation 2, Formulation 3 and Formulation 4 were smaller than expected which is below than 5µm. However, only Formulation 2 was indicated in stable formulation and clearly seen compared to other formulations under SEM test. Result obtained for UV-Vis test was observed Formulation 1, Formulation 3, and Formulation 4 has absorbed only UV-C region with indicate value of increment. Meanwhile, Formulation 2 has absorbed UV-B region that prevent skin from cancer risk. Therefore, Formulation 2 shows good absorption and exhibit high potential to be further developed and use as sunscreen in cosmeceutical products.

Keywords—Sunscreen; microemulsion; *Melaleuca cajuputi* essential oil; nonionic surfactant

I. INTRODUCTION

Nowadays, people more focus on appearance looking and cleanliness. Usage of cosmetics product for examples lipsticks, hair dyes, nail polish, cleansing cream, toothpaste, face mask

and ointment seem routine work [1]. The word “cosmetics” is derived from “cosmetae”, roman slaves whose bathe men and women in perfume. To discover the invention of cosmetics have to back to early 10 000 B.C. At that time, Egypt men and women already using scented oil to clean and soften skin as well as mask body odour. The Egyptians are also using oils and cream to protect themselves against hot sun and dry wind. The Egyptians learn the art to distill essential oils thousands years ago because they believed that the pleasant fragrance is essential as a sign of holiness in Egyptians belief. That is the beginning of perfumery industry. Around 3000 B.C., China had been using cosmetics products and continuous by Persia (Middle East), India, Japan, European country and others. So now, cosmetics products become well known since it can bring cleanliness, beauty and pleasant smell [2].

As time pass by, consumers expect the cosmetics products reach the high-performances levels and meet esthetic standards among others in mass market. To be outstanding, more and more technique and better formulation cosmetics products have been discovered to meet the consumers' requirement. Hence, different types of cosmetics vehicle types have been discovered such as solutions, emulsions, surfactants, preservatives and others. Solutions are the most fundamental cosmetic vehicles which water as major ingredient can serve as function of solvent. Besides that, emulsion is essential form in cosmetic products.

Emulsion is a type of disperse system that contains two or more immiscible substances which one substance is suspended or dispersed throughout another substance due to their mutually incompatible characteristics. So, emulsifying agents are often include in emulsion and function as dispersing agent,

spreading agents, solubilizer and others. Emulsion can be differentiated into a few types, which are oil on water (O/W), water in oil (W/O) and multiple (W/O/W) emulsion, hydrogels, microemulsion and nanoemulsion [3].

Microemulsion is specific system in which the emulsifying agents (surfactants) are optimally adapted to the oil and water phase and hence show the maximum interfacial activity. It is clear, thermodynamically stable and isotropic mixture. Microemulsion can be considered as small-scale of emulsion which droplet type dispersions either is oil in water (O/W) or water in oil (W/O) with a size range of 5-50 nm in each drop radius. Some of the advantages associated with microemulsion as delivery systems were improvement in solubilization, enhanced absorption due to surfactant induced permeability, increased penetration of hydrophilic, hydrophobic, amphiphilic substances, increased mobility and favorable modification of therapeutic activity [4].

The difference between emulsions and microemulsion is the emulsions unstable in thermodynamics and lead to phase separation but microemulsion is thermodynamically stable. It is because microemulsion has a lot of smaller droplet size than emulsion, so when mixing of one phase to another phase in the form of large amount of small droplets, large dispersion entropy arising. Besides, the introduction of surfactant into the interfacial layer of microemulsion contributes to favourable entropic system too. The large reduction of surface tension accompanied with significant favourable entropic change giving negative value of Gibbs free energy. Thus, microemulsion is thermodynamically stable [5]. In this research, the essential oil of *Melaleuca cajuputi* was extracted and used for formulation of microemulsion.

Melaleuca cajuputi is a type of Malaysia local plant which vernacular name is gelam or kayu putih. It has a synonym name is *Melaleuca leucadendron* and belong to family *Myrtaceae*. When the leaves of *Melaleuca cajuputi* were scrapped, it produced a "tea-tree smell" and that is the sources of cajuputi oil, a kind of essential oil. It has medicinal antiseptic, anti-inflammatory, antibacterial functions [6] which are potential to be skincare products. Besides that it also performs well in antioxidant [7], active cytotoxicity effect [8] and antibacterial [9, 10].

The microemulsion from *Melaleuca cajuputi* essential oil have not being discovered its sunscreen effect by combined with nonionic surfactant and this becomes the concerning issues in this research. Seeing that Malaysia's climate is primarily tropical, it remains hot and humid throughout the year and the sunlight is evitable. However, the utilization of *Melaleuca* oil and other botanicals could be remedies in sunburn recovery [11]. Hence, *Melaleuca cajuputi* which may potentially to be anti-sunscreen products is preliminary studying in this research by combining the co-surfactant (olive oil) and nonionic surfactant TWEEN 20, TWEEN 80, SPAN 20 and SPAN 80.

II. MATERIALS AND METHOD

A. Materials

All chemicals used in this research were commercially purchased from supplier as follows: TWEEN 20, TWEEN 80, SPAN 20, SPAN 80 and olive oil (Sigma-Aldrich); Petroleum Jelly (Vaseline); *Melaleuca cajuputi* essential oil (original). All the chemicals were used as supplied without purification.

B. *Melaleuca cajuputi* Essential Oil Extraction

Essential oils were extracted at MARDI (Malaysia Agriculture Research and Development Institute) at Telong, Bachok, Kelantan Darul Naim. In this research, the method used at MARDI was steam distillation method since the method was easy to control and the purification was proven. We used the fresh leaves from *Melaleuca cajuputi* on the same day. In steam distillation, plant material placed in a stiller. Water was flow into the tank and heated into the vessel and steam was sent through a coil into the stiller. As vapor passes through the plant material, the essential oil vaporized and released from the leaves. The essential oil saturated vapor rises out through refrigerated tubing, where it condenses and was drained off. Calcium chloride anhydride was added to the essential oil after the steam process to absorb the air in the solution.

C. Construction of Ternary Phase Diagram

The ternary phase diagram had been constructed to determine the stable ratio of the mixing system. The nonionic surfactant used was TWEEN 20, TWEEN 80, SPAN 20 and SPAN 80 and the co-surfactant used was olive oil. By calculation the different ratio of each component in every point of phase diagram, a total 5g of samples were prepared. After calculation each of the composition, the three components were mixed together in a small test tube according the ratio calculated. The homogenization process was then carried out. The vortex was used at maximum speed for a few minutes until it turned into milky solution and homogenized. Homogenization is to make sure the mixture is mixed well and homogenized [12].

After mixed by vortex, the test tube samples were placed in centrifuge to separate the phase of the mixture. The main function of centrifuge is to separate the three composition clearly by using the gravity forces. Centrifuge was used to rotate the test tube around a fixed axis which driven by electric motor and applying force perpendicular to the axis. So, the phases were separated. The centrifuge used can occupy 16 test tubes at once time. It was operates at 4400 rpm speed within 5 minutes. If less than 16 test tube mixtures is prepared, even number of test tubes have to be prepared to put in centrifuge evenly and balance the operation of centrifuge. After 5 minutes centrifugation, the test tube was removed from it and put in water bath 25°C. This method is to allow the temperature of mixture is reached equilibrium.

When the test tube was taken out to evaluate and determine the phase of each samples. The phases were determined with the aid of polarized lamp. The test tube was put under the polarized lamp to obtain clearer view and the phases were evaluated. The sample formed as one phase will have one

colour of mixture, but the two phases samples will have two layers, same goes for three phases samples will present of three layers. So, the phases of each mixture were recorded in phase diagram and the phase diagram is constructed.

The every point ratio was repeated to determine the phases. Then, the completed phase diagram was constructed. There are four different types of phase diagram consist of different kinds of nonionic surfactant. The nonionic surfactant used includes TWEEN 20, TWEEN 80, SPAN 20 and SPAN 80 in constructing the phase diagram while all the co-surfactant used is same, which is olive oil.

D. Preparation of Emulsification Process

In the emulsification process, the emulsion were mixing and shearing until the particles were suitable size to be stable. The surfactant used was TWEEN 20, TWEEN 80, SPAN 20 and SPAN 80 and co-surfactant is olive oil. Water was used as water phase because it is universal. The formulation of the microemulsion is according to the intersection point of phase diagram constructed. The thickener used was Petroleum Jelly. *Melaleuca cajuputi* essential oil was used as additives substances in the microemulsion to give pleasant odour and acts as potential sunscreen agents.

The microemulsion was prepared and the formulation was chosen according the 3 phases region from ternary phase diagram because it is the most stable region. Both of the oil phase and water phase were heated in the water bath till above 80°C. The water bath was used to prevent overheating and the thermometers were put inside the two phase's beaker to take the accurate reading of temperature [13].

To construct the oil-in-water system, the oil phase was slowly poured in water phase. Then, the microemulsion was continuing heat above 80°C to make sure it is homogenized as colloid system. Then, the microemulsion was removed from heat sources and proceed to homogenization. Homogenization is to break down the particles inside the microemulsion into smaller size and make it evenly dispersed in the system. So, the longer time taken for homogenization, the microemulsion can break into smaller size and more stable.

Next, three drops of *Melaleuca cajuputi* essential oil was added when homogenization. The microemulsion formed at the final stages were pleasant milky colour. Lastly, all the emulsifying samples were kept in dark cupboard to prevent the samples from contaminate and prevent react to light, heat or shaking. The samples were observed how long it can sustain.

E. Stability Test

The stability of samples was observed every day. Any physical changes such as colour changes, separation of phases, and aggregation of samples were considered unstable samples. The stability of samples can be influenced by the degradation of bacterial, the reaction with air and the unstable mixture condition between surfactant and non-surfactant.

F. Morphology of Sample Surface

After obtained the most stable formulation of microemulsion, the morphology of sample surface was observed under optical microscope and scanning electron microscope (SEM). For optical microscope, the specific glass slide and film sheet were rinsed with distilled water and acetone to prevent the contamination of other particles. A little amount of microemulsion was put on the film sheet and covered onto glass slide. Then, it was put at the optical microscope for observation.

The sample used in SEM must be in powder form without any moisture. So, drying the sample is vital to prevent breakage of SEM. The sample preparation was dried by using a graded series of ethanol followed by drying agent, 1,1,1,3,3,3-Hexamethyldisilazan (HMDS) [14]. Then, the samples were proceeding to high vacuum and coated with gold. When sample became powder form, it then proceeded to observe under SEM. A clear view of sample morphology was observed under SEM.

G. UV Spectroscopy

UV Spectroscopy was carried out to test the sunscreen effect of the microemulsion. Firstly, the microemulsion was used to determine the UV peaks before exposed to sunlight and the date was recorded. When exposed to sunlight, a bag of ice was put in basin to reduce the volatile percentage from bottle. After exposed to sunlight for 4 hours, the sample was run again by using UV Visible Spectroscopy to detect the adsorption. The absorption was recorded. This method was carried at the noon time, 12 pm to 4 pm at one of the open space in University Malaysia Terengganu.

III. RESULT AND DISCUSSION

A. Ternary Phase Diagram

The construction of ternary phase diagram is to construct the most stable formulation to obtain equilibrium phase with different types of nonionic surfactant using TWEEN 20, TWEEN 80, SPAN 20, and SPAN 80. By using the ternary phase diagram, each vertices means three components for surfactant, co-surfactant and water.

In every ternary phase diagram, one phase region is known as isotropic because this regions having equal value of possessions in all direction and uniformity in all orientation. The two phases and three phases regions are known as anisotropic region. It means these two regions are directional reliant [15].

Compared with other phases, three phases area is the most stable region because it has diverse orientation that is anisotropic. Besides that, after centrifugation, the composition of three phases mixture still able to separate into three different layers. It means that all three components still able to perform their own characteristics and properties well.

Three phases samples will be the most stable among the other phases samples. Figure 1, 2, 3 and 4 showed total four ternary phase diagram constructed based on different nonionic surfactant that are TWEEN 20, TWEEN 80, SPAN 20 and SPAN 80.

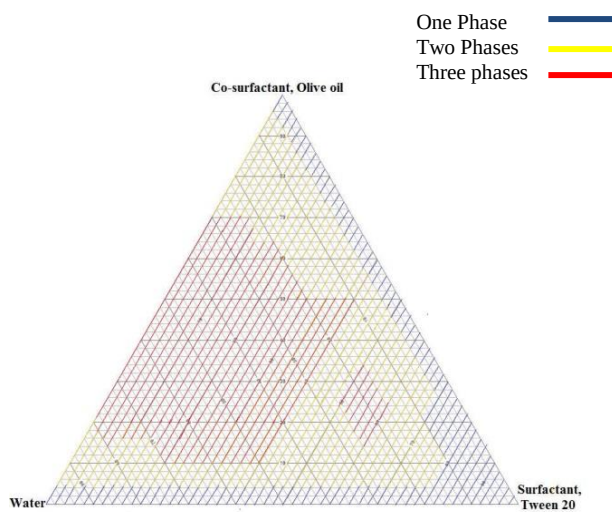


Figure 1. Ternary phase diagram of nonionic surfactant, Tween 20 with co-surfactant and water

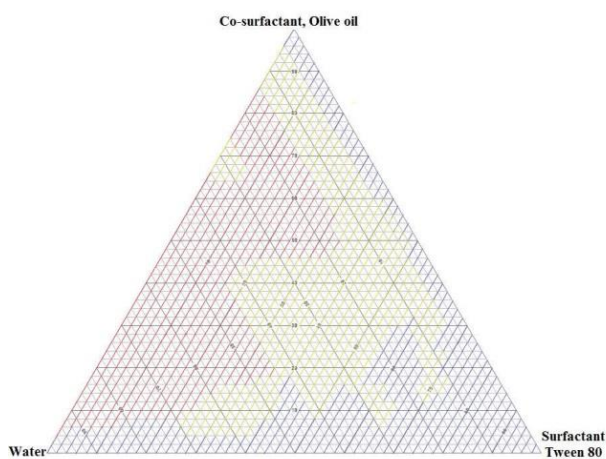


Figure 2. Ternary phase diagram of nonionic surfactant, Tween 80 with co-surfactant and water

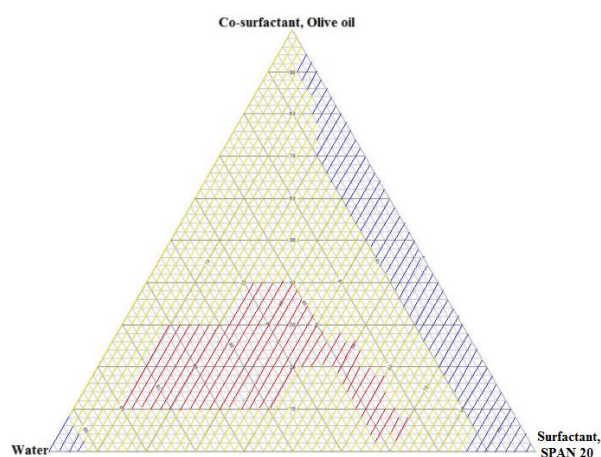


Figure 3. Ternary phase diagram of nonionic surfactant, Span 20 with co-surfactant and water

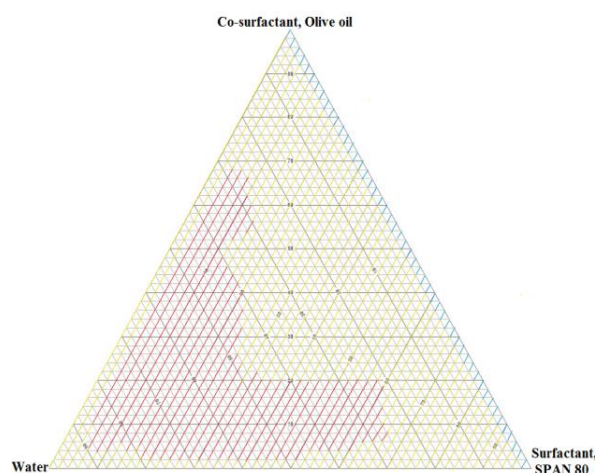


Figure 4. Ternary phase diagram of nonionic surfactant, Span 80 with co-surfactant and water

B. Emulsification Process

Emulsification process had been done according to the standard method and by referring to the ternary phase diagrams constructed. Four samples of 10 grams were prepared and kept in the cool dark place for a month. This is the most essential division where its guide to great constancy of colloidal medium. In this study, the microemulsion formed is oil-in-water (o/w) types of microemulsion as during the preparation, the oil phase consist of four different nonionic surfactant, olive oil and petroleum jelly (thickener) were poured into the water phase after both phase have been heated to 80°C. This type of microemulsion is classified as simple microemulsion where it just consists of one level of emulsification process.

In the oil-in-water system, the oil phase was slowly poured in water phase and the microemulsion was continuing heat above 80°C to make sure it is homogenized as colloid system. The microemulsion then was removed from heat sources and proceeds to homogenization to break down the particles inside the microemulsion into smaller size and make it evenly dispersed in the system. So, the longer time taken for homogenization, the microemulsion can break into smaller size and more stable.

The findings revealed that the freshly prepared microemulsion were milky white in colour for Formulation 1, 2 and 4 and yellowish white in colour for Formulation 3. Table I and Table II showed the formulation used to make the microemulsion samples.

TABLE I. FORMULATION OF MICROEMULSION SAMPLES

Material	Formulation			
	1	2	3	4
Surfactants	TWEEN 20 26%	TWEEN 80 30%	SPAN 20 28%	SPAN 80 10%
Olive oil	24%	20%	22%	10%
Water	50%	50%	50%	80%
Petroleum Jelly	3g	2 g	2 g	2 g
Essential oil	3 drops	3 drops	3 drops	3 drops

TABLE II. MASS OF EACH COMPONENT FOR MICROEMULSION SAMPLES

Material	Formulation			
	1	2	3	4
Surfactants	TWEEN 20 1.82g	TWEEN 80 2.40g	SPAN 20 2.24g	SPAN 80 0.80g
Olive oil	1.68g	1.60g	1.76g	0.80g
Water	3.5g	4.00g	4.00g	6.40g
Petroleum Jelly	3g	2 g	2 g	2 g
Essential oil	3 drops	3 drops	3 drops	3 drops

C. Stability test

The stability of samples was observed every day. Any physical changes such as colour changes, separation of phases, and aggregation of samples were considered unstable samples. Stable microemulsion is establish if the emulsion do not shows any physical changes including colour changes, separation of phases, aggregation or coalescence in the period. Full attention had been laid on the microemulsion in order to detect even a small change. Small changes will make the microemulsion fall into unstable microemulsion classification. The stability of microemulsion samples was observed by naked eyes. All the observation was recorded in Table III.

TABLE III. THE STABILITY OF THE MICROEMULSION SAMPLES

Durations / Formulations	Day 1	Day 2	Day 3	Day 5	Week 1	Week 2
Formulation 1	√	√	√	√	√	√
Formulation 2	√	√	√	√	√	√
Formulation 3	√	√	√	√	√	√
Formulation 4	√	√	√	√	√	√

√ Stable
X Unstable

D. Morphology of Sample Surface

Scanning Electron Microscope (SEM) test was carried out to observe the real morphology of microemulsion formulation. The water in emulsion was removed first since SEM test only can detect dry sample. Several series of ethanol (35%, 50%, 60%, 80%, 90% and 100%) were prepared and only 1 per 3 of emulsion filled into a vial. Then, emulsion was diluted with prepared series of ethanol and homogenized by Vortex mixer until mixed well. Next, the emulsion and water were isolated by centrifuge machine and dried with hexamethyldisilazane (HMDS).

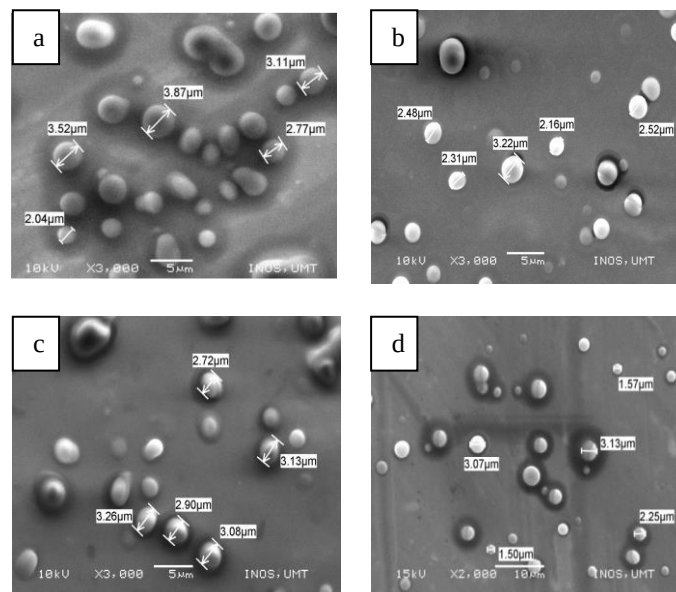


Figure 5. Morphology of microemulsion formulation under SEM (a) TWEEN 20, (b) TWEEN 80, (c) SPAN 20, and (d) SPAN 80.

Based on the Figure 5, all the particle sizes that observed were within the micro-size range. This properties obtained will ease the delivery and transportation on skin, hence it is easily absorbed by the human skin [16]. It has been clearly shown that the size droplet of oil in water microemulsion mostly at average 1.50-3.90 μm . The clearer results can be seen by adjusting the magnification.

The range size for (a) TWEEN 20 was from 2.04 – 3.87 μm while (c) SPAN 20 from 2.72 – 3.26 μm . However there was not clearly seen due to the wet condition sample and

becomes too oily. Meanwhile the size for (b) TWEEN 80 and (d) SPAN 80 was from 2.16 – 3.22 μm and 1.50 – 3.13 μm respectively. It also has been shown that stabilization occurred due to its uniformity and evenly distributed within the sample.

Formulations that were not fully dried and becomes oily were detected by observed the formation of dark circle around each of droplet and may not be clearly seen. Formulation (b) TWEEN 80 is better than formulation (a) TWEEN 20, formulation (c) SPAN 20 and formulation (d) SPAN 80 for SEM test. This is because sample is more stable, can be clearly seen and contributes favorable results compared to other formulations.

TABLE IV. THE ABSORBANCE VALUE FOR SUNSCREEN TEST

Formulation	Wavelength, (nm)	Absorbance		Increment
		Before	After	
Formulation 1	206.6	-	1.1281	1.1281
Formulation 2	286.2	0.4804	0.5329	0.0525
Formulation 3	235.4	-	0.1954	0.1954
Formulation 4	251.4	1.0547	1.2215	0.1668

E. Sunscreen Test

The main objective for sunscreen test is to determine the tendency of absorption ultraviolet (UV) radiation. This test was carried out with all formulations were exposed to sunlight. Then, the absorption peak value before and after exposed to sunlight was measured using UV-Visible spectroscopy. Water was used as solvent because of less absorbed sunlight and will record proper value absorption. Table IV was the results based on their increment value.

From the results obtained, all formulations showed positive results in absorption of UV radiation. Formulation 1, formulation 3 and formulation 4 were absorbed UV-C which range from 200-280 nm. So, the microemulsion formed can only absorb the harmful UV-C but not UV-A or UV-B. However, UV-C is unable to reach to earth atmosphere surface because it would absorb by ozone layer. Hence, currently UV-C will not harm the human skin [17,18]. However, formulation 2 was indicated tendency to absorb UV-B radiation at 286.2 nm which range from 280-315 nm and gave increment value was 0.0516. Both UV-A and UV-B radiations are very dangerous for human health which is can damage DNA and cause serious illness such as skin cancer [19]. Chemical constituent that has this UV radiation inhibitor property is chemical substances which contain carbonyl group such as phenanthrene [20]. Hence, it is believed that surfactant from Tween can be used as UV-absorber.

CONCLUSION

Microemulsions had been used as a vehicle for skin preparations since a long time ago [21]. In this research, the most stable ternary phase diagram which consists of surfactant, co-surfactant which is olive oil and water by comparing four phase diagram which using from different nonionic surfactant, TWEEN 20, TWEEN 80, SPAN 20, and SPAN 80 were constructed.

The region of the liquid crystal and micelle formation also had been discovered and plotted under the microscopic test. All samples of microemulsion had been fabricated from the phase diagram due to its potent liquid crystal whereas had been subjected to stability test.

This research aimed to produce emulsion from the most stable phase diagram had been accomplished where all formulations were showed good in stabilization process after stored in dark place in several weeks. However, only formulation 2 from non-ionic surfactant TWEEN 80 shows better result for SEM test which indicated stable formulation. This is because the morphology of each droplet microemulsion can be clearly seen under SEM test and it size becomes near to nanoemulsion. Besides, formulation 2 has better equal size of particles, more solidify and more stable compared to morphology of other formulations.

The formulation 2 of microemulsion had tendency to absorb the ultraviolet radiation especially in UV-B that prevent from lead to skin cancer compared to other formulations only absorb UV-C radiation. This is because of chemical properties from *Melaleuca cajuputi* essential oil that gives the main role act as sunscreen due to the carbonyl group such as phenanthrene. So, within nano size range of particle would be advantages for the system with extremely small droplet size which can increase penetration effect, highly interfacial area and capable to produce UV absorber to give sun protection [22].

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