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Pseudo-ternary Phase Behaviour of Palm-Based Microemulsion Insecticides[†]

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This research aimed to characterize microemulsion system of palm methyl ester (PME) and water/ mixed surfactants (Tween® 80 and Dehydol® LS 2)/ 1-propanol via a pseudo-ternary phase behaviour study. The microemulsion system with a hydrophilic-lyphophilic balance value of 12.33 was prepared using the constant composition method. The phases included an emulsion, a gel/viscous phase and a transparent microemulsion which were tested at different temperatures to examine their stabilities. Microemulsion samples were evaluated to obtain their colloid characteristics, such as particle size, viscosity, pH and conductivity. The optimum formulation of palm-based insecticide consisted of 48% water, with 20% PME, 20% mixed surfactants and 12% 1-propanol. The particle size of the microemulsion was 84.20 nm. The pH value was 6.6, the conductivity was 0.00951 µS/cm, and the viscosity was 20.44 cP. The results indicated that different amounts of surfactants and co-surfactant in the emulsion exhibited different phase behaviour in the pseudo-ternary phase diagram. Palm-based microemulsion potentially could be an alternative to replacing petroleum-based solvents in insecticides.

Key words: Pseudo-ternary phase; microemulsion; palm-based insecticides; palm methyl ester

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Insecticides have become important items to protect agricultural products and sites, in order to control pests that transmitting diseases such as dengue fever and Zika viruses [1]. Applied insecticides should be toxic only to the target organisms, renewable and environmentally friendly. Unfortunately, in most cases, insecticides may kill non-target organisms and pollute the environment [1, 2]. This situation occurs because more than 60% of conventional insecticides use petroleum or petroleum by-products as the main ingredient in the formulation [3]. Palm oil, as well as its by-products and oleochemicals, has become an alternative to replace petroleum-based solvents in insecticides because it is a renewable resource and biodegradable. Thus, palm oil and its derivatives, such as palm fatty acid methyl ester (PFAME) which also known as palm methyl esters (PMEs), have a potential to be utilized as the oil phase in a microemulsion study for practical applications [3].

Microemulsions are defined as systems containing a mixture of three components, i.e., a polar phase (usually water), a nonpolar phase (typically oil), and a surfactant, leading to isotropic and thermodynamically stable solutions [4, 5]. Also, they are also defined as oil-in-water (O/W) or water-in-oil (W/O) systems which are transparent, thermodynamically stable dispersions of two or more immiscible liquids wherein the dispersed phase consists of small droplets ranging in size from 10 to 100 millimicrons [4-16]. Besides, microemulsions system offer better efficacy in the insecticide formulation for controlling and killing the target insects because of the smaller size of particles allows the active ingredient to penetrate through insect's abdomen and cell membranes [17, 18]. The primary objective of microemulsion research is to determine the conditions under which the surfactant solubilizes the maximum amount of water and oil [19]. Besides, a pseudo-ternary phase diagram must be constructed to characterize a microemulsion system that has been developed.

The pseudo-ternary phase diagram provides information on the boundaries of different phases as a function of the composition variables such as temperature, and more crucial structural organization can also be inferred. The different aspects, the behaviours of phases and changes in the volume fraction of various phases of the system can be determined from the pseudo-ternary phase diagram [20]. The selection of the surfactant is also important because it is well established that a large amount of two immiscible liquids can be brought into a single

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phase by the addition of a suitable surfactant or mixed surfactants [21]. The selection of the surfactant and co-surfactant is crucial to creating a microemulsion in the ternary phase diagram. Generally, non-ionic surfactants are selected because they offer good cutaneous tolerance and, lower irritation potential and toxicity [22]. The tendency of a pseudo-ternary system to form microemulsion upon addition of a co-surfactant has been reported [23]. Surfactants and co-surfactants can reduce surface tension and increase the flexibility of an interfacial film [20]. The selection of PME as main oil ingredient as it has a high solubility rate in water compared to petroleum oil.

The experimental techniques to characterize microemulsions include conductivity via conductivity meter, viscosity via viscosity meter, and pH measurement via pH meter as well as particle size measurements via the dynamic light scattering method. Each technique provides useful information to determine the optimum formulation of palm-based microemulsion insecticides. The viscosity of the solution gives first-hand details on its internal consistency [24]. Based on Bancroft's rule, the conductivity measurement can predict which phases in the emulsion system is the continuous phase, i.e., either the water phase or oil phase. The pH measurement is important to determine the safety of insecticides when intact with human skin. Meanwhile, dynamic light scattering is one of the most useful techniques used to detect and measure the particle size and distribution of colloidal dispersions, emulsions, microemulsion, polymers, micelles, and proteins. Therefore, the particle size and distribution of palm-based microemulsion insecticides are determined by the dynamic light scattering technique. The droplet size is crucial for determining the internal point of deposition and how deep a droplet or particle will penetrate the respiratory system by inhalation.

The paper aimed to determine the optimum formulation of palm-based microemulsion insecticides by using a pseudo-ternary phase diagram with a mixture of water and PME/ mixture of non-ionic surfactant/co-surfactant—the microemulsion characterized by measuring pH, conductivity, viscosity and particle size.

MATERIALS AND METHODS

Materials

Palm fatty acid methyl ester (PFAME) also called palm methyl ester (PME), and Emery Oleochemicals (M) Sdn. Bhd. (Selangor, Malaysia) supplied Dehydol® LS 2EO (DLS 2). The non-ionic surfactants, Tween® 80 (polyoxyethylene sorbitan monooleate) was purchased from Cognis Oleochemicals (M) Sdn Bhd (Selangor, Malaysia). The purity of the surfactants ranged from 99.5% to 100% (w/w). The co-surfactant, 1-propanol was purchased from Bumi-Pharma Sdn Bhd (Selangor, Malaysia). Water was deionized and filtered.

Preparation of Microemulsion Insecticide

Tween 80® was chosen as the primary surfactant (S1) with Dehydol® LS2 as the secondary surfactant (S2). Water and oil (PME) with a weight ratio of 75:25 were added into a 10 ml screw-cap glass tube. The mixture of surfactant was prepared by mixing both surfactants at an S1 to S2 weight ratio of 70:30. The mixture of the surfactant was then added into the samples at weight percentages of 5%, 10%, 15%, 20%, 30%, 40%, and 50% (w/w). The co-surfactant, 1-propanol was then added into the samples to bring the total weight to 100%. The sample was vortexed using a vortex mixer (Scientific Industries Inc., USA) at 1800 rpm for 5 minutes. Then, samples were incubated in a water bath at 50°C for two hours and equilibrated at ambient temperature (25°C) for 24 hours. The formation of microemulsions and other phases was observed by the polarized light sheet. Then, all of the samples were tested for their thermal stability. Each sample was vortexed again before being kept in an oven for two weeks at 45°C. The samples were observed on day 1, day 2, day 14 and day 30 [3,23].

Construction of Pseudo-ternary Phase Diagram

The pseudo-ternary phase diagrams consisting of PME, water, Tween® 80: DLS2, and 1-propanol systems were constructed using the optimum formulation that was selected based on the best stability of the system concerning the thermal stability test. The pseudo-ternary phase diagrams were constructed using Chemix School v3.50 software (Arne Standnes, Norway). Four different diagrams were constructed which included, day 1 at 25°C, day 1 at 45°C, day 7 at 45°C, day 14 at 45°C and day 30 at 45°C, based on the observations from the thermal stability test [3].

Characterization of Microemulsion

Based on the ternary phase diagrams, the samples that were transparent, low in viscosity and stable were selected and labeled as microemulsion samples. The samples were characterized concerning the physicochemical properties of the microemulsion [24]. Specific conductivity (in µS/cm) of microemulsions was determined using a conductivity meter (SG3-ELK, Mettler Toledo, Columbus, OH). Rheometer (MCR 300, Anton Paar) was used to measure the viscosity of the samples. The measurement was carried out at ambient temperature (25°C) using a double gap measuring system with a shear rate of 100, (model DG26.7/T200). The pH analysis of formulation was carried out using pH Tutor Bench meter (Eutech Instrument, UK) at 25°C. The particle size of the microemulsion solution was analyzed by zetasizer instrument (Nano Zs, Malvern Instrument Ltd., UK). The measurements were carried out using analysis software (DTS Nano version 5.03) at 25°C.

RESULTS AND DISCUSSION

Phase Diagram and Appearance

There are a wide variety of structures and phases formed by mixing PME, water, surfactant, and co-surfactant. The primary aim of the phase behaviour study is to determine the conditions under which the surfactant solubilizes the maximum amounts of water and oil [15]. The best microemulsion formulation should have solutions that are clear, transparent, low in viscosity and exhibit non-birefringence properties [5]. Visual inspection was made after the samples were prepared to classify the samples that had a microemulsion system. The microemulsion samples were identified because they appeared clear, transparent and flowable. Meanwhile, the emulsion samples seemed to be milky, and the gel samples appeared as liquid crystal; i.e., the liquid did not flow. In Figure 1, the appearances of the samples are shown: (a) a transparent microemulsion samples, (b) a milky emulsion samples and (c) gel samples with a liquid crystal region. All of these categories were plotted on the pseudo-ternary phase diagram using Chemix 3.50 software.

Four different ternary phase diagrams were constructed based on the thermal stability tests conducted, which included, observations at 25°C and 45°C, for day 1 until day 30 as shown in Figure 2 (a), (b), (c), and (d). The surfactants were applied at different concentrations from 5% up to 50% (w/w). Tween® 80 and DLS2 were selected as the surfactants because they offer lower HLB values, which were 12.33. Mixed surfactants were employed in the samples to attain some intermediate properties or synergetic effects [25]. Blending the surfactants may increase the miscibility of oil and water. The ratio of oil to water (PME to water) was selected to be 25:75 because this study emphasizes systems with large amounts of water (or aqueous phase). The tendency of a pseudo-ternary system to form a microemulsion upon the addition of 1-propanol as a co-surfactant has been reported [24]. The co-surfactant, 1-propanol acts by further reducing the interfacial tension between the oil and water phases. Moreover, 1-propanol also interacts with surfactant monolayer to increase the flexibility of the interfacial film [4].

Figure 2 (a) to Figure 2 (d) shows the ternary phase diagrams of PME and water/ Tween® 80 and DLS 2/ 1-propanol systems with increasing concentrations of surfactants. The ternary phase diagram consists of three phase regions, i.e., isotropic liquid region (L1), liquid crystalline region (Lc), and multiphase region. Based on the ternary phase diagram, the green region is known as the microemulsion region, L1, and a clear, transparent and low viscosity sample is found. In the red region (Lc), liquid crystal formation is observed, which occurs due to the excess hydrophilic content. Meanwhile, the blue region determines the multiphase region where most of the samples are not stable and produce birefringence properties.

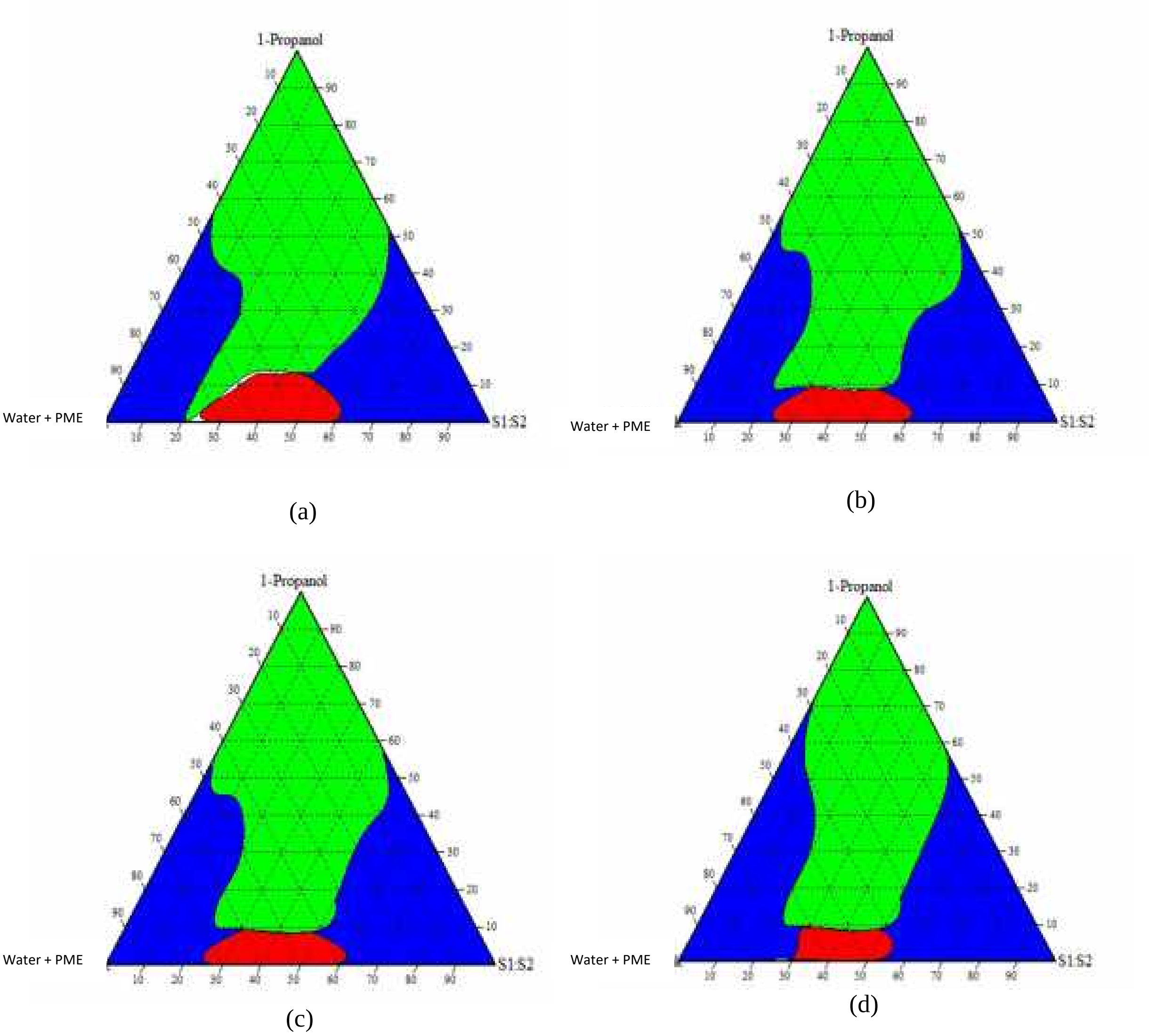
Based on the ternary phase diagrams in, Figure 2 (a) to Figure 2 (d), the L1 region is reduced by the increasing temperature. This observation occurs due to the microemulsion samples changing to two layers emulsion, and the microemulsion samples become unstable at the higher temperature. Hence, they tend to separate to form a multiphase region. The Lc region is the most extensive in Figure 2 (a), and this region does not destabilize much when the temperature increases to 45°C. Besides, the crystal liquid regions get larger with the addition of surfactant. This behaviour shows that the temperature-dependent phase behaviour of the ternary system is a result of the interplay between the lower miscibility gap of the oil (PME)-S1: S2 mixture and the upper miscibility gap of the water-S1: S2 mixture. Basically, at lower temperatures, both of the non-ionic surfactants (Tween® 80 and DLS2) are mainly soluble in water, and they are soluble in oil at high temperatures. Thus, as the temperature increases, both of the non-ionic surfactants change from hydrophilic to hydrophobic.

Based on Figure 2 (a), the green region is the most extensive part as sample formed a stable clear and transparent solutions in one phase. Based on the ternary phase diagram (a), at 20% of mix surfactant, most of the sample formed a clear and transparent solution. It happened due to the formation of micelles is sufficient to dissolve oil and water. Micelles formed when the concentration of surfactant higher than the concentration of critical micelles, CMC. Starting from 30% to 50% of mix surfactant, most of the sample developed liquid crystal phase. The higher percentage of mix surfactant used, sample likely to become liquid crystal solution. Besides, with the addition of 1-propanol, the tendency for the sample to form microemulsion is higher. The co-surfactant help to reduce the surface tension and increase the solubility between surfaces [26].

The microemulsion formulations were then further selected based on the results from the ternary phase diagram. Based on the ternary phase diagram in Figure 2 (a), the samples located in the green regions selected for further characterization to find the optimum formulation of palm-based microemulsion insecticides.

(a) (b) (c)

Figure 1. Samples of the (a) clear and transparent microemulsion, (b) the milky emulsion, and (c) the gel sample.



(a) (b) (c) (d)

Figure 2. Ternary phase diagrams at different temperatures for 30 days. Where (a) is the ternary phase diagram in day 1 at 25°C, (b) is the ternary phase diagram in day 1 at 45°C, (c) is the ternary phase diagram in day 14 at 45°C and (d) is the ternary phase diagram in day 30 at 45°C.
Indicator: Green is a cisotropic liquid region (L1), red is the liquid crystalline region (Lc), and blue is the multiphase region.

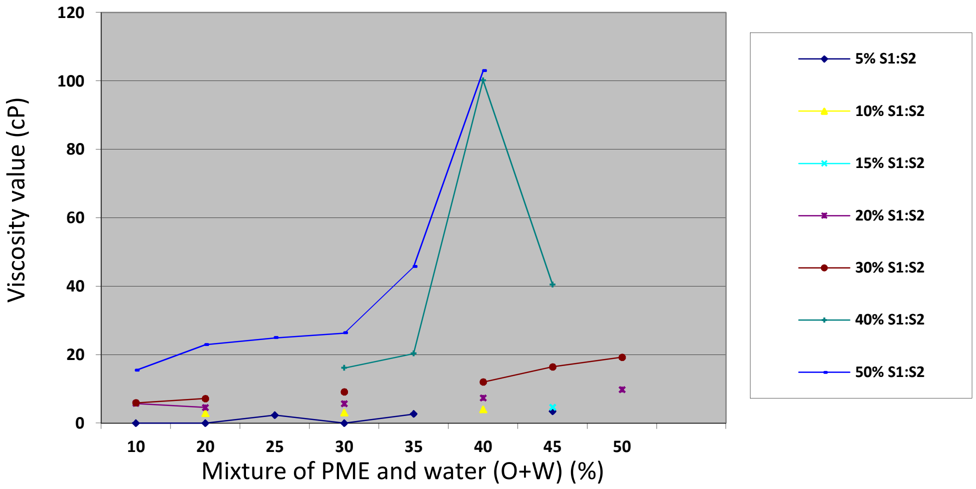


Figure 3: Graph of viscosity value against percentage of PME and water mixture for all samples.

pH Measurement

The aim of the pH test was to determine the pH that is suitable for use with humans and the environment. Human skins have a pH of 5.5 and samples shall have pH value between 5 to 7. Based on the pH measurement, the microemulsion samples having pH values of approximately 4.24 ± 0.5 to 6.50 ± 0.5. The sample with low pH value had more tendency to corrode the aerosol container and not suitable to be used for in-house usage. The sample which formed pH value in between 5 to 7 selected for a conductivity test.

Conductivity

Conductivity test conducted to determine continuous phase in samples. Based on the conductivity test, mean of conductivity value was 0.0004357 μS/cm ± 1.05. The conductivity values for all samples were lower than 1μS/cm as it was affected by the type of surfactants used. In this research, Tween® 80 and Dehydol LS2 were selected as surfactants for preparing the microemulsion formulation. Both surfactants were non-ionic surfactant and non-polar. No charge carried by both surfactants. Based on the result, the sample with a higher volume of water gives higher conductivity value. Based on conductivity value, in 10% wt/wt water content, an increase in the conductivity was observed, and a sharp increase in the conductivity was observed at 30% wt/wt water. The increase in conductivity at higher water concentrations is most likely caused by a transition from an oil-continuous microemulsion system to a water-continuous microemulsion system [4]. This situation happened due to the transition of continuous-oil microemulsion system to continuous-water microemulsion system.

Viscosity

Based on the viscosity measurements, with a 20% mixed surfactant, there are plenty of samples that show low viscosity properties. All the samples exhibited Newtonian flow behaviour, which is the behaviour of microemulsions [4]. Also, the viscosity value can be increased slightly when the water concentration is increased, or the system becomes an oil/water type [18]. The increase amount of water makes the viscosity value decrease. Based on the results, at 10% to 30% of mix oil and water mixture, the viscosity value recorded low. This situation is driven by increased water content in the system as well as changes in microemulsion phase structure from sphere to asymmetric [27]. Based on the graph 1, viscosity value reached the highest at 40% and 50% of mix surfactants at 35% of oil and water mixture. The viscosity value drops drastically to higher water content. Therefore, a sample with 20% mix surfactant selected for further testing of the microemulsion.

Particle Size

Generally, microemulsions will have a particle size between 10 to 100 millimicrons and appear clear or transparent [6]. The mean droplet size and polydispersity index of palm-based microemulsion insecticides are measured by dynamic light scattering. Based on the results (Figure 3 a, b and c), the smallest droplet size was 11.85 nm ± 0.788, followed by 84.20 nm ± 0.931 and the largest droplet size was 117.6 nm ± 0.917. In Figure 3a, there was a broad peak observed due to the instability of the microemulsion sample. Meanwhile, in Figure 3c, a single peak of the particle size was observed but, the particle size of the microemulsion sample was too large. Because the particle size of a microemulsion

Table 1. Optimum formulation for palm-based insecticides and characterization of the optimum microemulsion formulation.

Optimum formulation	%	pH	Conductivity (µS/cm)	Viscosity (cP)	Particle size (nm)
Mix surfactants (Tween® 80: DLS2)	20	6.6	0.00951	20.44	84.20
PME	20				
Co-surfactant (1-propanol)	12				
Water	48				

should be less than 100 nm, the formulation with a ratio of 20/65/15 of mixed surfactant/ PME and water/ 1-propanol in which the particle size was 84.20 nm was selected as the optimum formulation (Figure 3b).

Based on the observations, when the concentration of the surfactant increased, an increase in the droplet size is observed. This occurs due to the spontaneity of the emulsification process increasing at higher surfactant concentration due to the excess penetration of water into the bulk oil, causing massive interfacial disruption and the ejection of droplets into the bulk aqueous phase. Droplet sizes of 11.85 to 117.6 nm are obtained with good size distribution, and the polydispersity index shows that all the formulations had a narrow size distribution (<0.2). Due to the low polydispersity index, and, nano-sized particles, the minimum composition of mixed surfactant/ PME and water/ 1-propanol used in this formulation was chosen as the optimum composition of palm-based insecticides. Thus, the optimum pH, conductivity, viscosity and particle size value are stated in Table 1.

CONCLUSION

The proper selection of the amount of surfactant, co-surfactant and oil is very essential for the development of the optimum compositions of microemulsion insecticides. The construction of a pseudo-ternary phase diagram is an important tool to assess the effect of different concentrations of surfactant on the oil and water held inside the emulsion system. Impressive results with the presence of the mixed non-ionic surfactant (Tween® 80 and DLS 2) in the formulation which supported the formation of microemulsions and turbid emulsion phases with no gel phase was produced. The results from the characterization methods (conductivity, pH, viscosity and particle size) were extremely useful for determining the optimum composition of palm-based microemulsion insecticides formulation. Hence, palm-based microemulsion insecticides had great potential to replace commercial insecticides by replacing the petroleum-based solvent with palm-based solvent, and to reduce medical issues as well as saving the environment while doing its main purpose which is to reduce insects transmitting diseases. Besides, it helps to reduce medical issues and save the environment, as well as to reduce insects transmitting diseases. This study will be beneficial in formulating a microemulsion system to be applied in the insecticides industry as well as in cosmetics and personal care products.

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