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Influence of Temperature on the Phase Behaviors and Techniques Toward Formation of Palm Oil Esters Nanoemulsion
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Palm oil esters (POEs), which are newly synthetic liquid wax esters with HLB value of 9.34, were proposed as a lipophilic phase for formation of nanoemulsion. Phase diagrams show domination of two-phase regions at all emulsification temperatures ranging from 30 to 80°C. Spontaneous and temperature-induced emulsification, high-shear and high pressure homogenization were utilized to form nanoemulsion. However, only high pressure homogenization successfully produced droplets sizes in the nano range. Thus, it was used to optimize the stability properties of POEs nanoemulsion. The manipulation of processing temperatures during the formation of emulsions could be used in lowering the droplets size of the emulsion.

Keywords: Nanoemulsion, palm oil esters, phase behavior, temperature

1. INTRODUCTION

Wax esters are esters of long-chain fatty acids with long-chain fatty alcohols.[1] Liquid wax esters are also known as unsaturated wax esters because of the unsaturation of their molecules and their liquid physical state of the mixtures of fatty alkyl esters moiety. Natural liquid wax esters such as sperm whale oil and jojoba oil are very expensive and limited in access. Therefore, the market is continuously in search of a replacement to fulfill the demand in cosmetics and pharmaceuticals industries.[2–4] In this work, palm-based wax esters are synthesized from refined-bleached-deodorized (RBD) palm oil and oleyl alcohol in our laboratory. Palm oil esters (POEs) with chain length of C28-36 possess high potential as a superior substitution to jojoba oil, which is widely used in commercial cosmetics.[5] These newly synthesized esters with high molecular weight are extremely stable substances and do not easily deteriorate.[6] This is on par with the industry’s interest to use ingredients that are non toxic, biodegradable, and that come from renewable sources.[7] POEs have the novel property of excellent wetting behavior without any oily feeling at interfaces, as well as other specialty characteristics such as good fat soluble properties and being non irritating.[8]

One possible way of utilizing POEs is to incorporate them into an emulsion or any possible colloidal forms. However, a preliminary study about the phase behavior must first be conducted as to acquire a better understanding of using POEs as a promising lipophilic phase. In this article, the phase behavior of POEs was reported using Span 20 as surfactant from 30 to 80°C in an attempt to establish a correlation between POEs and temperatures.

Nanoemulsions are transparent or translucent system mostly covering the size range of 50–200 nm.[9] The nano-size of the emulsion droplets offers them the characteristics feature of being easily permeates and absorbed by the skin.[10] Nanoemulsions are kinetically stable but very fragile systems by nature. The slightest sign of destabilization such as opaque and creaming easily appears as they are transparent and usually very fluid.[9,10]
Typical lipophilic phase such as medium-chain triglycerides (MCTs) or short-chain esters such as isopropyl myristate or isopropyl palmitate are relatively inefficient to be emulsified into nanoemulsions as compared to long-chain esters. This is because the lower the molecular weight, the higher the polarity of the oil and, hence, the higher the Ostwald ripening rate. Thus, the use of long-chain POEs in our work will provide better stability due to tremendous reduction of Ostwald ripening rate. Heavier alkanes were used to slow down Ostwald ripening as longer chain length promotes lower solubility and diffusibility of oil phase through the continuous medium. The different types of fatty acid moieties in POEs would also enable them to be superior as compared to single esters in terms of stability. Currently, there are very limited works on the use of palm-based esters for nanoemulsions formation in various fields. Therefore, the objective of this particular work is to study the potential of palm-based esters for the formation and the stability of the prepared nanoemulsions.

2. MATERIAL AND METHODS

2.1. Materials

The POEs were synthesized in the lab. Span 20 were purchased from Merck, Germany. Water used in this work was deionised water to avoid surface-active impurities.

2.2. Methods

2.2.1. Construction of Phase Diagram

Various ratios of POEs:Span 20 ranging from 0:100 to 100:0, with total weight of 0.5 g were weighed and vortexed in screwed-closure 5 mL glass sample tubes for 15 minutes. Then, it was stored in water bath at 30 ± 1.0°C unless stated otherwise as 40, 50, 60, 70, or 80 ± 1.0°C. Upon the addition of a single drop of water, the altered weight of the system was recorded and the mixtures were left at the desired temperature for 30 minutes and then vortexed for two minutes. The samples were then centrifuged at 5000 rpm for 10 minutes. Water was gradually added to the samples until it reaches 100 wt% composition. The phase behavior of the mixtures was determined through visual observation and any presence of liquid crystalline phase through the continuous medium. The different shearing rates ranging from 5000 to 30000 rpm and was homogenized for duration of 1 to 10 minutes. Emulsion flowed into the stationary stator and the moving shearing rotor acted as a cutter to ‘slice’ bigger emulsion droplets to smaller ones. The second part also varies the emulsification temperatures from 30 to 60°C for a minimal shearing duration of two minutes.

2.2.3. Preparation of Emulsion Using Low Shear Rate Emulsification

Emulsion was prepared using low shear rate emulsification with RW16 stirrer (Ika-Werke, Germany). Pre-emulsion was stirred at a stirring rate ranging from 200 to 1000 rpm at various durations from 0 to 600 minutes. In the second part, the emulsification temperatures were varied from 30 to 60°C, respectively, and for a minimal stirring duration of ten minutes.

2.2.4. Preparation of Emulsion Using High Shear Rate Emulsification

Emulsion was prepared using high shear rate emulsification with Polytron PT 3100 (Kinematica AG, USA). The study started with subjecting the pre-emulsion to different shearing rates ranging from 5000 to 30000 rpm and was homogenized for duration of 1 to 10 minutes. Emulsion flowed into the stationary stator and the moving shearing rotor acted as a cutter to ‘slice’ bigger emulsion droplets to smaller ones. The second part also varies the emulsification temperatures from 30 to 60°C for a minimal shearing duration of two minutes.

2.2.5. Preparation of Nanoemulsion Using High Pressure Homogenization

Emulsion was prepared using high pressure homogenization with NS1001L (Niro Saovi, Italy). Pre-emulsion was prepared before they were introduced to different pressures of the homogenizer ranging from 200 to 1000 bar and for repeating cycles from 1 to 10. A combination of impingement, implosion and shear forces disrupt the fluid stream reducing it to a finer emulsion. The two-phase mixture was subjected to strong conversion of pressure to kinetic energy which then breaks the dispersed phase into small droplets.

2.2.6. Measurement of Droplet Size Distribution

The particle size distribution (PSD) of samples was measured using Nanophox particle size analyzer (Sympatec, Germany) which implies diffusion method using dynamic light scattering (DLS) with Argon laser (λ = 488 nm). The measurement was conducted using Photon Cross Correlation Spectroscopic (PCCS) principle. The background and sample were measured for 600 s by diluting one drop of the emulsion with 10 ml of aqueous phase. The measurement was performed at 25.0 ± 0.5°C using the freshly prepared emulsions. The droplet size distributions were integrated using non-negative least-square (NNLS) algorithm evaluation mode resulting in an optimization of the recognizable fine structure of the droplet collective. The results were presented as the volume mean distribution (VMD).

2.2.7. Stability

The stability of nanoemulsion was assessed by measuring the droplet size as a function of time. For each of the graph
r³ versus time, a trendline was drawn and the value of the slope for each of the linear stroke represents the Ostwald ripening value. The lower the value of the Ostwald ripening indicates a more stable system of the nanoemulsion.

3. RESULTS AND DISCUSSION

3.1. Phase Studies

The ternary phase diagram of POEs/H₂O/Span 20 was constructed at 30, 40, 50, 60, 70, and 80 ± 1.0°C in Figure 1a–1f. Isotropic regions, 1p, which was found along the POEs and Span 20 axis in all the phase diagrams, could possibly be due to high solubility of Span 20 in POEs. The isotropic regions however were observed to uptake only 3–7 wt% of H₂O at phase diagrams constructed at 30, 40, 50, 60, and 80°C whereas phase diagram constructed at 70°C could uptake up to 15 wt% of H₂O. It is suggested that this scenario is probably due to better mixing of POEs and Span 20 to form a clear or transparent liquid at the surfactant-oil apex line when the temperature is increased. However, the constructed phase diagrams did not show any liquid crystalline region. Although it has been proposed before that higher concentration of POEs will ease the formation of micelles, nevertheless, the polar group is inadequate to compensate the long carbon chain length of POEs moiety to reduce the interfacial tension and uptake maximal solubilization of oil to form liquid crystalline phase.

Two-phase regions which dominated the phase diagrams simply showed that the mixtures were unable to mix well and therefore had formed creamy heterogeneous liquids. Two types of two-phase were observed in the phase diagrams. Two-phase with separated ‘creamy-clear’ layers of liquids or known as the creaming phenomena, 2p¹, was reduced in phase diagrams which were constructed from 30 to 60°C and was not found in phase diagrams constructed at 70 and 80°C. The other type of two-phase observed was two-phase with separated ‘clear-clear’ layers of liquids which were identified as 2p². Both the upper and lower liquids appeared as clear solution that was immiscible to each other. 2p² regions were found to increase as the temperature of the phase diagrams was increased from 40 to 80°C. As both 2p¹ and 2p² were unstable mixtures, subsequent continuous or sufficient amount of energy and proper mixing protocol could lead to homogeneous creamy mixtures.

Three-phase regions, 3p, were found only in the phase diagrams constructed at 30 and 40°C. Apparently, the 3p region was reduced from phase diagrams constructed at 30 to 40°C and was not found in phase diagrams constructed at 50°C or higher. 3p was recognized as separated three “clear-clear-clear” layers of liquids. Thus, it was clearly shown that increment in temperatures has affected the behavior of the systems. Heat has promoted a better mixing which comes from better self-assemble of molecular interactions. Although it did not improve the systems to form larger isotropic regions, it has eliminated 3p region at 50°C and higher. However, temperature alone may not be sufficient for the system to be well homogenized. Further mechanical energy could be introduced to form a better well-mixed mixture.

3.2. Pre-Emulsion Preparation

As the rate of addition was increased from 1 mL/min to 30 mL/min, the droplet size of the pre-emulsion increased. Addition of 1 mL/min gave the lowest droplet size distribution with 2125 nm. Therefore, 1 mL/min was suggested as the rate of addition of water into oil phase to form smaller droplet size distribution of emulsion.
3.3. Formation of Emulsion Using Low Shear Rate Emulsification at Various Time and Temperatures

Figure 2 shows the effect of time with respect to different stirring rates. Stirring at 400 rpm produced the lowest droplet size distribution followed by 600, 200, 800, and 1000 rpm. However, the data also illustrated that the longer the duration was taken for emulsification, the lower the droplet size distribution of emulsion formed. If a system is allowed to stir to accumulate sufficient kinetic energy during the formation of emulsion, then a smaller size distribution of emulsion droplets will be produced. Subsequently, a system would generate better stability as smaller droplets are less attracted to each other due to weaker Van der Waals forces between them. However, as low stirring rate was still preferable as it produced smaller emulsion droplets. Nevertheless, the system designed in this study was incapable of producing very fine emulsion droplets at nanometer range.

Figure 3 shows the effect of stirring rates with respect to different temperatures. The duration of emulsification was maintained at 10 minutes to evaluate the effect of temperature toward formation of emulsion at low shear rate emulsification at a minimum time. Systems showed reduction of droplet size distribution with increasing temperature from 30 to 60°C. System at 60°C has produced the lowest emulsion droplets, 920 and 780 nm at 800 and 1000 rpm, respectively. These could be due to the utilization of energy has firmly absorbed or utilized by emulsion droplets to be more kinetically energetic and thus induced less occurrence of aggregation. It is clarified that nonionic surfactant with higher HLB value is preferred to emulsify emulsion as the temperature increases in order to maintain the actual HLB value of the system.\[16\]

3.4. Formation of Emulsion Using High Shear Rate Emulsification at Various Time and Temperatures

Figure 4 shows the formation of fine emulsion droplets at various shearing rates ranging from 5000 to 30000 rpm at different time intervals. The results illustrated that the lower shearing rates at 5000, 10000 and 15000 rpm produced finer emulsion droplets with droplet size distribution ranging from 180 to 462 nm as the duration of homogenization increases. On the other hand, the higher shearing rates at 20000, 25000 and 30000 rpm produced coarser emulsion droplets with the droplet size distribution ranged from 281 to 1566 nm. Similar time course profile which show either a size reduction period or an initial size

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**FIG. 2.** Droplet size distribution of emulsion produced using low shear rate emulsification from 200 to 1000 rpm at various durations from 0 to 600 minutes.

**FIG. 3.** Droplet size distribution of emulsion produced using low shear rate emulsification from 200 to 1000 rpm at various temperatures from 30 to 60°C.

**FIG. 4.** Droplet size distribution of emulsion produced using high shear rate emulsification from 5000 to 30000 rpm at various durations from 1 to 10 minutes.
reduction period followed by leveling-off period with respect to increase in homogenization intensity as depicted in the former results has been reported.\footnote{17} This could be explained from the hydrodynamic point of view. The equilibrium emulsion droplet size (UEEDS) at the leveling-off period for 10 000 and 15 000 rpm shearing rates were the critical points where the turbulent eddies became so small that their energy is lost as heat through dissipation and not being utilized to break the dispersed phase into smaller droplets any further.\footnote{18} Subsequently, shearing rates at 20000 rpm and higher which show a different profile of increased droplet size with time course could be clarified by the incapability of high mechanical shearing rate to generate the turbulent eddies of a size smaller than those of the critical points and the heat accumulated caused the emulsion droplets to aggregate and hence, reduction in long-term stability. The use of nonionic surfactant, Span 20 which was less stable at high temperature may not be suitable to be processed at higher shearing rate of 20000, 25000, and 30000 rpm without any heat transfer mechanism.

In Figure 5, the effect of shearing rates with respect to different temperatures toward emulsion droplets has been carefully studied. The experiment was performed in a water bath in order to control the heat flow and to maintain the desired processing temperature. It was assumed that the heat generated from the emulsification process was equivalent to heat release by the water bath to the environment. Figure 5 shows that at any stage of emulsion regardless of the shearing rates, the droplet size distribution increased as the temperature was raised. The finest emulsion droplets were from the systems at 30° C which ranged between 280 to 480 nm whereas the coarsest emulsion droplets were from the systems at 60°C which ranged from 656 to 1788 nm.

3.5. Formation of Emulsion Using High Pressure Emulsification at Various Pressures and Cycles

Table 1 shows the effect of the pneumatic pressure toward the droplet size distribution of each of the emulsion system. It evidently depicts that by increasing the pressure, the size of the emulsion droplets reduced greatly. The emulsion showed 180 nm at 200 bar and decreased to 100 nm after 1000 bar was applied to the system. By applying pressures, it deflocculates clusters of primary fat globules and disperses agglomerates uniformly.\footnote{19} From the additional studies, it was seen that by utilizing the high pressure homogenizer to create smaller dispersion emulsions, it could almost demolished the effect of different surfactant used in the study (data not shown). During the formation of emulsion, droplet size distribution of the emulsion which undergoes high pressure homogenization was influenced by the rates of breakage and coalescence in the homogenizer.\footnote{15}

Table 2 reveals that by adding cycles to the system, the size of the emulsion droplets could be reduced. Similar effect was found when the cycles of homogenization was increased\footnote{20} The size reduced drastically from the first to sixth cycles before a plateau size was observed from seventh cycle onward. It is suggested that the turbidity of the emulsions decreased with increasing number of cycles which indicated finer emulsion droplets.\footnote{21}

3.6. Stability of Nanoemulsion

Nanoemulsion is only kinetically stable and therefore, they can be referred the term “approaching thermodynamic stability” due to their sizes which induced long-term physical stability.\footnote{9,22} In order to determine the kinetic stability of the prepared nanoemulsion, Ostwald ripening rate was derived from the slope of the linear relationship graph of radius\footnote{3} versus time according to the Lifshitz-Slesov-Wagner (LSW) theory up to 8 weeks. Figures 6 and 7 show the long-term stability of nanoemulsion, which were subjected to different pressures and cycles as a function of storage time for 30 weeks. Table 1 showed that increasing pressure would lead to a more stable nanoemulsion as indicated by lower slope values of Ostwald ripening rate.

<table>
<thead>
<tr>
<th>Pressure (bar)</th>
<th>Initial radius, $r_0$</th>
<th>Ostwald ripening rate, $\omega$</th>
</tr>
</thead>
<tbody>
<tr>
<td>200</td>
<td>180</td>
<td>$2.55 \times 10^5$</td>
</tr>
<tr>
<td>400</td>
<td>123</td>
<td>$1.42 \times 10^5$</td>
</tr>
<tr>
<td>600</td>
<td>115</td>
<td>$6.09 \times 10^4$</td>
</tr>
<tr>
<td>800</td>
<td>112</td>
<td>$5.30 \times 10^4$</td>
</tr>
<tr>
<td>1000</td>
<td>100</td>
<td>$3.01 \times 10^4$</td>
</tr>
</tbody>
</table>

FIG. 5. Droplet size distribution of emulsion produced using high shear rate emulsification from 5 000 to 30 000 rpm at various temperatures from 30 to 60°C.
Nanoemulsion which was subjected to 200 bar was experiencing other instabilities as the trendline was no longer linear after ten weeks. This was not in agreement when nanoemulsion is often described as stable toward flocculation and coalescence due to high kinetically stable system.\(^{[22]}\) The phenomenon was probably due to less uniformity in sizes after one cycle at 200 barr and that causes accelerated diffusion of oil droplets through continuous phase in a non-equilibrium system. It is also mentioned that flocculation may participate in the instability of nanoemulsion during long-term storage process.\(^{[16]}\) On the other hand, Ostwald ripening rates also show decrement as the number of cycles increased at a constant 200 bar as summarized in Table 2. Cycles 1 and 2, however, gave no readings of Ostwald ripening rates as the graph was not in a linear relationship after approximately 7 or 8 weeks. This phenomenon indicated that the dominant instability that took place after the linear relationship was not Ostwald ripening but other breakdown processes such as flocculation and creaming. Ostwald ripening is shown to be the main destabilization mechanism of the phenoxy dextran emulsion when the particle volume varies linearly with time for duration of four months and the volume distribution function gave no variability.\(^{[11]}\) A comparison between the droplet sizes in Tables 1 and 2 shows that by subjecting the nanoemulsion to nine or ten cycles, the formulations could be stabilized and could achieved the same particle sizes as the nanoemulsion subjected to 800 or 1000 bar for a cycle. The increasing diameter of the emulsion droplets was due to the increasing diffusion of the POEs droplets. Furthermore, the differences in the ripening rates were probably caused by the differences in the molar volumes of water and POEs. The results were also in agreement with LSW equation indicating that the larger the emulsion droplets, the larger the Ostwald ripening rate. It signifies that the larger the solute molecule, the faster the droplet size changes as a result of Ostwald ripening.

### 4. CONCLUSIONS

POEs/H\(_2\)O/\(\text{Span 20}\) phase diagrams produced at different temperatures from 30 to 80°C showed different phase behaviors. Nanoemulsion could be formed by using various techniques from low to high energy emulsification methods. Low shear rate and high shear rate emulsifications did not successfully produced emulsion in the nano range, however, with the combination of low and high pressure emulsifications, it has produced nanoemulsion droplet as small as 78 nm with long-term stability. From the study, it showed that regardless of type of surfactant, high pressure homogenization is very useful to be used as secondary method to form finer emulsion droplets. Temperature also plays an

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**TABLE 2**

<table>
<thead>
<tr>
<th>Cycle</th>
<th>Initial radius, (r_0)</th>
<th>Ostwald ripening rate, (\omega)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>180</td>
<td>–</td>
</tr>
<tr>
<td>2</td>
<td>144</td>
<td>–</td>
</tr>
<tr>
<td>3</td>
<td>123</td>
<td>(1.28 \times 10^5)</td>
</tr>
<tr>
<td>4</td>
<td>118</td>
<td>(1.21 \times 10^5)</td>
</tr>
<tr>
<td>5</td>
<td>112</td>
<td>(1.19 \times 10^5)</td>
</tr>
<tr>
<td>6</td>
<td>103</td>
<td>(8.94 \times 10^4)</td>
</tr>
<tr>
<td>7</td>
<td>99</td>
<td>(7.99 \times 10^4)</td>
</tr>
<tr>
<td>8</td>
<td>98</td>
<td>(4.76 \times 10^4)</td>
</tr>
<tr>
<td>9</td>
<td>98</td>
<td>(1.67 \times 10^4)</td>
</tr>
<tr>
<td>10</td>
<td>97</td>
<td>(1.25 \times 10^4)</td>
</tr>
</tbody>
</table>

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**FIG. 6.** \(r^3\) of nanoemulsions as a function of time for samples produced using high pressure emulsification at various pressures.

**FIG. 7.** \(r^3\) of nanoemulsions as a function of time for samples produced using high pressure emulsification at various cycles.
important role in emulsification especially when a nonionic surfactant system was used. Temperature affects not only the viscosity, but also interfacial tension in the case of non-ionic surfactants or more generally interactions forces inside the system.

REFERENCES