Effect of Mixed Emulsifier on the Physicochemical Properties of Avocado Oil Nano-emulsions

Tzung-Han Chou, Daniel Setiyo Nugroho, and Chia-Hua Liang

***Abstract*—The objective of this study was to provide insights into the synthesis and characterization of oil-in-water nano-emulsions system prepared using mixed surfactant to induce nano-colloid formation. Nano-emulsions (NE) were obtained by pre-homogenization continued with ultrasonication of herbal drug loaded avocado oil, hydrogenated lecithin (HL), and d-α-tocopheryl polyethylene glycol 1000 succinate (TPGS) in the aqueous phase. The NE constituted with vary of surfactant ratio, surfactant to oil ratio, and oil content to investigate the physicochemical properties and storage stability. Thus, the morphology NE was examined and performed in regularly spherical shape distributed within 33–270 nm. Where the forming of micelle was increasing the polydispersity index and confirmed by TEM analysis. The results indicated that particle size of the nano-emulsions was majorly affected by the surfactant ratio and surfactant concentration. Moreover, the presence of TPGS decrease the phase transition region until completely eliminate it and make the NE become thermally stable. To investigate the stability of NE, visual observation was done and found that the unstable NE run into phase separation less than 24 hours. In contrast, the stable NE was found to be stable more than 365 days where found affected by the presence of micelle. The current result suggests that mixing TPGS and HL enhance the NE stability and induce micelle formation significantly. On the other hand, TPGS perform significant improvement in stability of NE and establish it be promising lipophilic compound carrier.**

***Index Terms*—Hydrogenated lecithin, mixed micelle, mixed surfactant, nano-emulsions, TPGS.**

I. Introduction

Chemical instability, poor water-solubility and dissolution properties of several lipophilic compound regularly limit their bioavailability and delivery efficacy. Various strategies to improve their physicochemical characteristic have been investigated, including the use of nano-emulsions, nanoparticles and, liposomes [1], [2]. The preparation of suitable nano-emulsions system for food, cosmetics, and drug delivery attracted much attention because its advantage to encapsulate, protect and, release lipophilic compound. However, in many applications, the physical and chemical stability, formation, and functional attributes of nano-emulsions can be improved by mixing two or more emulsifiers [3], [4]. Nevertheless, there is currently a relatively poor understanding of mixed emulsifier systems impact on nano-emulsions formation and properties.

The current improvement of surfactant study exploring the surfactant mixture phenomena which possesses a high surface activity and enhances micellar properties [5]. When two or more emulsifiers are mixed together in solution or in an emulsion, they may interact with each other through various types of molecular interactions, such as hydrophobic, electrostatic, or hydrogen bonding. The fact that surfactant mixtures are regularly used in practical applications is because of it known to lower the critical micelle concentrations (CMC) value and synergistically improved mixture properties of surfactant interaction [6]. CMC refers to a concentration where all additional surfactants in the system aggregates and micelles are formed in the dispersion. The aggregation of surfactant molecules to forms micelles is expected to prolong the shelf life, and lower the viscosity of the nano-emulsions. The related issue has been reported by Lu et al, 2019 which investigated the mixed SDC/TPGS mixed micelle to improve the solubility of Zinc phthalocyanine [7]. Furthermore, Singla et al, 2019 also examined the influence of Pluronic F108 and its mixed micelles with surface active ionic liquids (SAILs) to get deeper understanding about quercetin solubilization phenomena [8]. Their finding was successfully improving the drug loading, solubility and, drug delivery performance, but there still limited understanding about the behavior of mixed micelle stability improvement mechanism.

For further learn about mixing surfactant, hydrophile-lipophile balance (HLB) could be one of important parameter to investigate their mixing behavior. HLB number is often used to describe their solubility and partitioning characteristics. Surfactants with low HLB numbers (2–6) partition into the oil phase and stabilize water-in-oil emulsions, whereas surfactants with high HLB numbers (8–18) partition into the water phase and stabilize oil-in-water emulsions. In this study, nonionic TPGS and amphoteric HL used as the emulsifiers. TPGS known to have high water-soluble emulsifier with HLB number 14, where HL with HLB number 6 make it high oil-soluble and pretend to separate in emulsion form. However, HL known as a good emollient with amphoteric charged in physiological condition, this special surfactant characteristic will help to control the drug release in oral or topical administration. Combining TPGS and HL as oil in water emulsifier intended to improve their physicochemical characteristic performance since TPGS known has poly ethylene glycol (PEG) head group that induce stearic effect and provoke the forming of micelle [9]. Furthermore, opposite HLB value expected to increase

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partitioning characteristic not only in water but also in oil situation who will help to get a better encapsulation efficiency and good stability.

Nano-emulsions were produce using high-energy preparation method by mechanically homogenized than continued with ultrasonication to provide enough energy to form small and narrow distributions of particle size. The physicochemical characteristics of these emulsions were investigated by transmission electron microscopy, dynamic light scattering, differential scanning calorimetry, and fluorescence polarization. Furthermore, the effect of the nano-emulsions constituents on their physical properties and storage stability was also evaluated.

II. Materials and Method

A. Materials

The avocado oil was purchased from Storino's Quality Product, USA. D-α-tocopheryl Polyethylene Glycol 1000 Succinate (TPGS) was purchased from Eastman, UK. Hydrogenated Lecithin (HL) were supplied from Merck KGaA (Germany). Uranyl acetate was purchase from Electron Microscopy Science, Britain. 1,6-Diphenyl-1,3,5-hexatriene (DPH) was obtained from Sigma-Aldrich Chemie GmbH, Steinheim, Germany. Double distilled water produces from Sartorius, Millipore Ultrapure, was used for all preparations of nano-emulsions.

B. Preparation of BSFL Oil Nano-emulsions

Initially, the avocado oil, surfactants and aqueous phase were pre-heating in 80ºC in 4 mins then homogenized at 15,000 rpm for 2 mins with D-500 homogenizer (Wiggen Hausser, Germany). Further, the dispersion sample was sonicated under a power of 60 W at 45ºC for 20 mins with an ultra-sonicator (Misonix 3000, USA). Nano-emulsions synthesized with avocado oil, 1% and 2% and the total emulsifier consisted of surfactant ratio (SR) of HL/TPGS at 0/100, 25/75, 50/50, 75,25, 100/0 with surfactant to oil ratio (SOR) of 30%, 50%, and 60% in the aqueous phase. After ultrasonication, 4 mL sample was obtained and each formulation repeated three times (n=3) at least.

C. Characterization of Nano-emulsions

Nano-emulsion average particle size (APS) and polydispersity (PdI) measurement were carried out using a dynamic light scattering (DLS) Zeta Plus Analyzer (Brookhaven Instruments Corporation, USA) and followed by zeta potential (ZP) measurement. 1 ml of the prepared nano-emulsions were added to the cuvette which diluted for 20 times in pure water at room temperature. Hydrodynamic particle size and polydispersity index were obtained from automatic cycle calculation in 3 mins, where the zeta potential was measured in 10 cycles of calculation by phase analysis light scattering. The measurement was made in triplicates for each sample and reported in mean ± standard deviation. The size of the nano-emulsions particles was also confirmed by transmission electron microscope (TEM) analysis.

The morphology of the nano-emulsions was visualized under operation of a transmission electron microscope (HITACHI H-7500, Japan) at 100 kV. Approximately 5 µL of nano-emulsions was dropped onto 3 mm carbon film coated copper grid to gain the particle deposition. Further, nano-emulsions were negatively stained with uranyl acetate and kept for 3 min at room temperature and subsequently dried before observation.

Thermal analysis of nano-emulsions was carried out using differential scanning calorimeter (DSC), DSC 1 instrument with the STARe system (Mettler Toledo Inc., Switzerland). 20 µL samples were placed in aluminum pans and then sealed, while 20 µL water-sealed pans used as a reference. The sealed pans were placed in the DSC chamber with an initial temperature of 25°C. Samples were cooled to −10°C at 5°C/min and then heated to 80°C at 5°C/min. Every measurement was done with three cooling-heating cycles and made in triplicate (n=3).

D. Fluorescence Polarization Measurement

The 1,6-diphenyl-1,3,5-hexatriene (DPH) was used as a fluorescence polarization probe for measuring intra-nano-emulsions molecular fluidity. Appropriate amounts of DPH was incorporated in the oil phase for each sample and analyzed using a fluorescence Spectrometer (Perkin Elmer LS 55, USA). The measurement of vertical and horizontal polarization was done in excitation wavelength of 360 nm (Ivv) and the emission wavelength at 450 nm (Ivh). The degree of polarization was obtained by the following equation from the average of 10 measurement cycles at 25ºC, where G = 1 is the built-in factor of the instrument. All the sample was repeated triplicate (n=3).

E. Stability Analysis of Nano-emulsions

To investigate the dispersion stability, all sample was kept in room temperature with low light intensity. Visual observation and DLS measurement were done every day for the first week, then followed for every seven days. The unstable nano-emulsions solution was judged by average particle size ≧ 800 nm, polydispersity index ≧ 0.6, and/or the bulk phase separation phenomenon such as flocculation and precipitates in the bottom of the bottle.

F. Statistical Analysis

All the experiments were repeated three times. Error bars represent the standard deviation, ± 20 for APS, ± 0.1 for PdI, and ± 2 for ZP allowed, respectively. The $p < 0.0001$ were calculated with one-way ANOVA using Design Expert 10 software. Numerical optimization carried out to get the optimum point for APS.

III. Results and Discussion

A. Particle Size, Polydispersity, and Zeta Potential Characteristic

The small size of nano-emulsions droplet and lower polydispersity index is one of the most significant parameters imparting to the colloidal stability and also provides high surface area to volume ratio. Keeping these factors in mind, two different surfactants, TPGS and HL, were assessed for their effect on droplet size. From Fig. 1, illustrates the average particle size (APS), polydispersity index (PdI), and zeta potential of avocado oil nano-emulsions with various surfactant ratios (SR) of HL/TPGS at different surfactant to oil ratio (SOR) and oil content obtained from DLS measurement. As depicted in Figure 1a, increasing SOR and surfactant ratio causes a decrease in the size of nano-emulsions. Thus, it was found that the APS significantly affected by SOR and surfactant ratio (P=<0.0001) rather than oil content (P=0.5272). This effect suggested that abundant amount of surfactant will provide a stronger reduced ability in surface tension and higher contact probability to interact with oil rather than low SOR. Such effect may tighten molecular distance, resulting in a decrease of particle size [10]. In the case of oil content, a higher amount of oil in nano-emulsions does not cost any noticeable change in particle size. This indicates that the nano-emulsions size is more significantly affected by the partition equilibrium of surfactant in solution by compared with oil content.

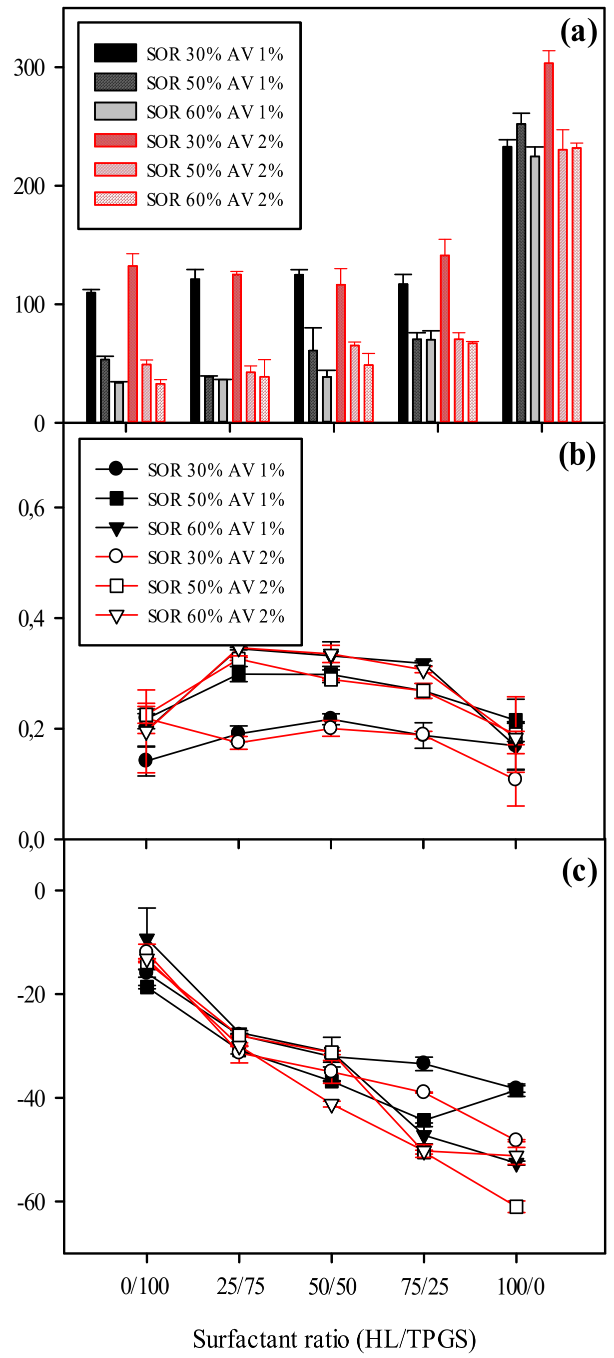

Fig. 1. Effect of surfactant ratios of HL/TPGS on (a) average particle size, (b) polydispersity index and, (c) zeta potential of fresh avocado oil nano-emulsions at SOR 30%, 40%, and 50%, with oil content 1% and 2%.

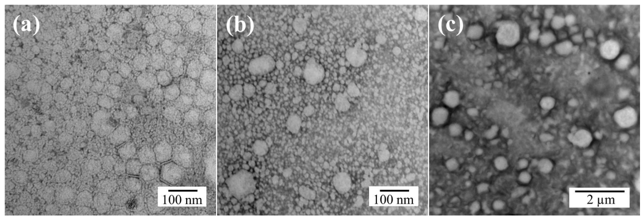

Fig. 2. TEM images of fresh avocado oil nano-emulsions prepared with SOR 60%, oil content 1%, and surfactant ratios of HL/TPGS for (a) 0/100, (b) 25/75, (c) 100/0.

As for surfactant ratio effect (Fig. 2a), the presence of TPGS was significantly maintain the particle in small size even in lowest TPGS concentration at surfactant ratio of HL/TPGS in 75:25. Appearance of TPGS can gives the bulky PEG head group in the HL nano-emulsions surface, inducing high curvature of particles [10]. It is interesting to note in Fig. 2b that the polydispersity index of nano-emulsions with mixed binary surfactants is larger than that of single HL or TPGS nano-emulsions. This phenomenon may be related with the miscibility between TPGS and HL. Besides, results of zeta potential (Fig. 2c) displays that an increase of HL amount in the nano-emulsions induces a decrease in the zeta potential. This may be due to HL used in this work is negatively charged.

B. Morphology Assay

The morphology which related to the size of the nano-emulsion was confirmed by TEM analysis. Fig. 1 shows the TEM images for fresh prepared nano-emulsions with various surfactant ratios of HL/TPGS as SOR = 60% and oil content =1%. The analysis demonstrated that the diameter of the particles was consistent with the data obtained by DLS. One can see that the existence of nearly spherical particles and the size distribution is different for each formulation. This indicates that avocado oil adding with HL and TPGS can form nanoscale colloids in water. Besides, the size of nano-colloids for pure HL is larger than that of those for TPGS and HL/TPGS = 75/25, suggesting that the incorporation of TPGS into HL nano-emulsions would induce the molecular rearrangement.

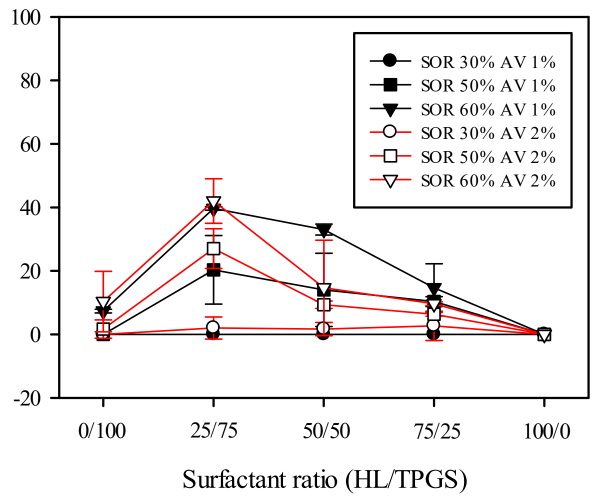

Fig. 3. Effect of surfactant ratios of HL/TPGS on mixed micelle formation intensity of avocado oil nano-emulsions at SOR 30%, 40%, and 50%, with oil content 1% and 2%.

A similar decrease effect of TPGS on size of Tween 80 nano-emulsions was also reported [11]. Generally, the bulky of PEG (2000) group can impart sufficient curvature of particles [10], but neighbor constituent interaction shall not be neglected in the nano-colloids. As will be confirmed in the following section, the sizes of avocado oil nano-emulsions visualized by TEM were virtually agreed with those determined by DLS.

Moreover, the forming of micelle become fore pronounce when TPGS was mixed with HL (Fig. 3). The result suggests that mixture of amphoteric surfactant and nonionic surfactant will strongly interact because of different electrostatic attraction between the headgroups [12]. Whereas, the hydrophobic interaction between the alkyl chains of the two kinds of surfactants in mixed solution make it easier to form micelles. As a result, these mixtures will probably lower the cmc value and increase in polydispersity.

C. Stability Study and Visual Observation

The physical stability of the avocado oil nano-emulsions was evaluated by visual observation to examined macroscopic appearance change during storage. Fig. 4a shows that the most transparent solution was in the surfactant ratio of 75:25, which gives very high storage stability until 365 days. Emulsion with high transparency has a low refractive index which related to the small particle involve inside the solution. Therefore, the transparency of the nano-emulsions has the same tendencies with the particle size trend. These phenomena are in the same agreement with other report [2] where combining of water-soluble surfactant and a water-insoluble phospholipid can result a single surfactant micelle, mixed micelle, or mixed dispersions. Furthermore, it was found that a micellar or mixed micellar formation will generate an isotropically clear solution [13].

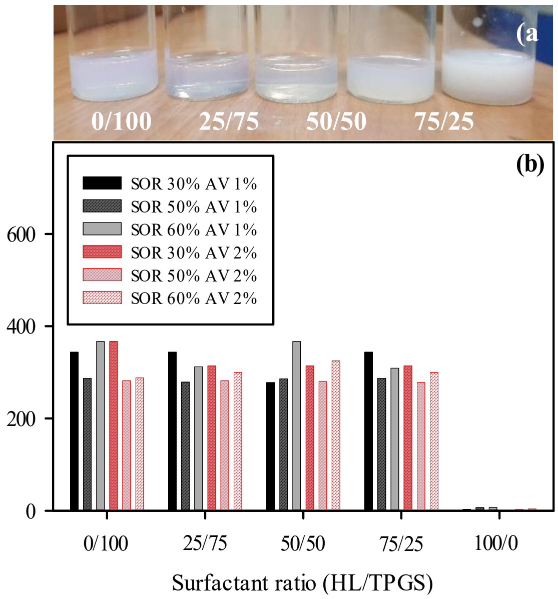

Fig. 4. (a) visual appearance of avocado oil nano-emulsions at SOR 50% and oil content 1% compared with (b) storage stable days. Error bars are standard errors from triplicates.

While HL use alone as a surfactant in the avocado oil nano-emulsions, white cream layer was observed in the surface of the solution after reach 2-7 days. (Fig. 4b). Although the HL nano-emulsions have the lowest zeta potential value, the huge size of particles was found and phase separation appeared. In most cases, the destabilization ofnano-emulsions dispersion is caused by the phase transition, gravitational force, and flocculation or Ostwald ripening [14]. Creaming is one of the frequent observed phenomena in this system because of the oil phase density is lower than the aqueous phase density. Moreover, HL known has low solubility in water which more likely acts as a hydrophobic surfactant. So that the oiling-off does not happen, which mean that the oil phase does not separate from the surfactant. It is proved that HL has an excellent performance to incorporate oil phase in their environment.

At fixed oil content and SOR, one can find that the endothermic curve of HL nano-emulsions exhibits larger size of phase transition area with the presence of TPGS. According to the report [15], the phase transition is belong to pure HL dispersions which implies that adding of TPGS would disturb the molecular packing of HL nano-emulsions. TPGS owning PEG head group may provide steric effect toward the neighbor molecule in the nano-emulsions.

By compared with Figure 5a and 5b, an increase of SOR induces a more obvious size of phase transition area. An increase of surfactant amount affects phase transition region which related to surfactant and avocado oil interaction. In the other hand, pure TPGS phase transition region appear in the temperature range of 28.7°C-41.3°C with melting point at 37.3°C (data not shown). Nevertheless, the endothermic peak of TPGS was not observed in the DSC thermograms of NE, suggesting a complete molecular dispersion of TPGS in both oil and aqueous phase.

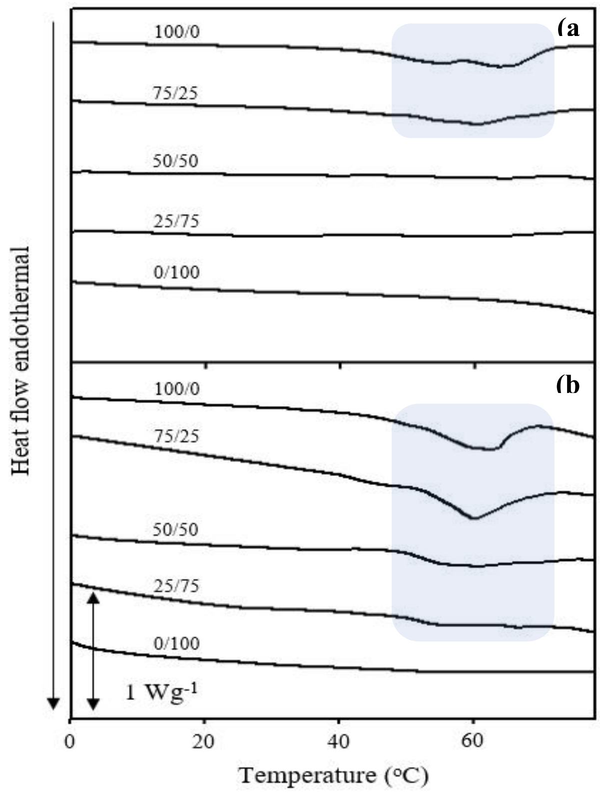

Fig. 5. Endothermal DSC thermogram of avocado oil nano-emulsions with different surfactant ratios of HL/TPGS at (a) oil = 1%, SOR = 30% and (b) oil = 1%, SOR = 60%.

Table I present the main phase transition temperature (ΔTm), enthalpy change (ΔH), and half-width transition temperature ($\Delta T_{1/2}$) for every recorded peak from DSC measurement. The result suggests that increasing TPGS concentration at SOR30% will cost decreasing ΔTm from initially 63.24ºC become 60.5ºC. Where the changes become pronounce at SOR 60% from 61.81ºC and reach 4.61ºC. This result may attribute to the creation of the less ordered structure or numerous lattice defects of the inner structure of the lipid matrix and/or the small size effect which could be explained by Gibbs-Thomson equations [16]. Thus, in low TPGS concentration, the interaction between TPGS and avocado oil is more dominant rather than the interaction with HL. Moreover, increasing SOR would make the surfactant interaction firmer. Furthermore, ΔH which belong to HL phase transition exhibit the same behavior even in high or low SOR which the presence of TPGS even in lowest concentration can reduce the phase transition region that make the NE become thermally stable.

TABLE I: ENTHALPY CHANGES (ΔH), MAIN TRANSITION TEMPERATURE (ΔTM), AND HALF-WIDTH TEMPERATURE (ΔT1/2) OF AVOCADO OIL NANO-EMULSIONS FROM ENDOTHERMAL DSC DATA

SOR %	SR (HL/TPGS)	ΔTm	ΔH (mJ)	$\Delta T_{1/2}$
30	0/100	-	-	-
	25/75	-	-	-
	50/50	-	-	-
	75/25	60.5	-1.01	5.31
	100/0	63.24	-4.92	8.65
60	0/100	-	-	-
	25/75	54.61	-1.1	4.11
	50/50	57.52	-4.11	6.81
	75/25	59.88	-5.27	4.27
	100/0	61.81	-12.4	5.36

In case of $\Delta T_{1/2}$, its clearly indicate that mixing TPGS and HL will decrease the phase transition temperature width which in the same evidence with sharpening the transition peak. This result is in the contrary with [17], which explain that bigger particle will crystallize at faster rates as compared to smaller ones. However, when mixing TPGS and HL will cost decreasing particle size and accelerate the phase transition rates.

D. Molecular Fluidity in Nano-emulsions

The fluidity of nano-emulsions membrane is one of the essential aspects that affect drug delivery performance. The high fluidity of a membrane may have good performance since its ability to squeeze and deform through the various physiological barrier [18]. The polarization of DPH inside the dispersion was found in the same value even in the different amount of surfactant ratio and oil content (Fig. 6). Increasing SOR make the polarization value of nano-emulsions decrease for all oil content 1% and 2%. This implies that the intra-nano-emulsions fluidity can be elevated by increasing surfactant amount. But oil content seems not to influence significantly on the molecular fluidity in avocado oil nano-emulsions.

E. Statistical Analysis and Optimization

The statistical analysis was performed to find the relation of independent variables: SOR, oil content, and the ratio of surfactant to the dependent variables: average particle size, zeta potential and, polydispersity index. In order to get understanding about mixing phenomena, user-defined design with 5 different points for surfactant ratio was carried out in this study. The ANOVA analysis obtained using two-factor Interaction (2FI) model with inverse transformation. The result suggests that obtained model showed an actual correlation between independent response and variable. Where, the p-value for APS, PdI, and ZP was found to be $p < 0.0001$, $p < 0.0419$, $p < 0.0001$, and the F-value was found to be 36.31, 2.65, 11.33, respectively (data not shown). This result implies that the entire model is significant and has a proper representation. Furthermore, there was only a 0.01% chance that an F-value this large could occur due to noise. Regression equations for each response variable, obtained from response surface methodology are mentioned in Eqs. (1)-(3):

$$\frac{1}{APS} = 0.014 + 6.2^{-3}A - 3.54^{-4}B - 7^{-3}C - 6.43^{-5}AB - 5.3^{-3}AC + 9.77^{-6}BC \quad (1)$$

$$\frac{1}{PdI} = 4.27 - 0.64A - 0.082B + 0.37C - 0.064AB - 0.12AC - 0.081BC \quad (2)$$

$$\frac{1}{ZP} = -0.034 - 2.7^{-3}A - 2.71^{-3}B + 0.016C + 1.74^{-3}AB + 3.2^{-3}AC + 2.99^{-3}BC \quad (3)$$

From Eq. (1) it was found that two factors (A and BC), affected the average particle size in positive direction. When increasing of surfactant in specific range of oil content and surfactant ratio amount will decrease the particle size. Thus, positive coefficient of C in Eqs. (2) indicates that increasing surfactant ratio will cost increasing of polydispersity. Furthermore, four factors in Eqs. (3) (C, AB, AC, and BC) will decrease the zeta potential value. Whereas, all negative coefficient in the equation indicates an opposite effect on independent variable.

The optimal condition for NE was determine using numerical optimization with targeting the minimum level of APS and ZP value and set in PdI to be lower than 0.3. The solution with maximum desirability value was selected as the optimized material composition. Thus, the predicted APS reaches at 33.84 nm with desirability of 0.998 and resulted in the composition of independent variable as follows, SOR 59.93%, oil content 1.002%, and HL/TPGS ratio 0.108/0.892, respectively.

IV. CONCLUSION

In the present study, avocado oil incorporated with mixing TPGS and hydrogenated lecithin was successfully prepared. The dispersion forms nearly spherical nano-emulsions with long-lasting storage stability by a homogenization-ultrasonication method. The physicochemical properties of BSFL oil nano-emulsions, including morphology, size, size distribution, zeta potential, viscosity, endothermic phase behavior, molecular fluidity of intra-particle was analyzedand their results depended on their formulation constituent such SOR and SR. The presence of TPGS can reduce sizes of nano-emulsions of HL nano-emulsions and disturb the molecular packing of HL nano-emulsions, resulting in an increase of molecular fluidity in nano-emulsions. Moreover, adding of TPGS would induce the forming of mixed micelle and prolong the storage stability. The information in this study will be useful for understanding the behavior of mixing surfactant and give a better design for preparing stable nano-emulsions dispersion for application in drugs, foods, and cosmetics.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

All authors were contributing in writing the paper, with additional work; Daniel S Nugroho conducted the research; Tzung-Han Chou and Chia-Hua Liang analyzed the data and deep reinforce the fundamental content; all authors had approved the final version.

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He continued working at PT. Rubberman Indonesia as a process engineer located in Bekasi, Indonesia. Currently, he pursuing a master's degree at National Yunlin University (NYUST), Yunlin, Taiwan started in 2018 with a major in chemical and material engineering. His research field was focused in interfacial and biotechnology laboratory under the supervision of Prof. Tzung-Han Chou.

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