Original Article

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Effect of the hydrophilic-lipophilic balance values of non-ionic surfactants on size and size distribution and stability of oil/water soybean oil nanoemulsions

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ABSTRACT

Objective: This study aims at investigating the effect of the hydrophilic-lipophilic balance (HLB) of non-ionic surfactants on physical property and stability of nanoemulsions. **Materials and Methods:** The nanoemulsions were prepared using the Microfluidizer. The oil phase consisted of 10% w/w soybean oil and 5% w/w surfactant(s). Types of nanoemulsions were confirmed by dilution test. The average droplet size and size distribution (polydispersity index [PDI]) were measured by dynamic light scattering Zetasizer Nano ZS™. The screening physical stability was done by centrifugation. Only the formulations with no phase separation were further evaluated for physical stability. **Results:** All nanoemulsions were single phase turbid liquids with no intrinsic creaming. The formulation with HLB 15 showed significantly larger droplet size followed by HLB 10, 9, 8, and 4.3, respectively. The PDI values were in range of 0.071–0.117. All nanoemulsions regardless of the HLB values showed that no significant change in droplet size and the Ostwald ripening rates was almost zero. **Conclusion:** The formulation with the mixed surfactants of HLB 8 seems to be promising for the future development of cosmetics because this nanoemulsion had the smallest droplet size which could enable efficient actives movement to the skin because of its remarkably high surface area.

Keywords: Hydrophilic-lipophilic balance, nanoemulsions, non-ionic surfactants, physical stability, soybean oil

INTRODUCTION

Nanoemulsion is a colloidal dispersion system that contains spherical droplets in the range of 20–200 nm in diameter, resulting in translucent or turbid liquid. Nanoemulsion is metastable and kinetically stable but thermodynamically unstable, in which Ostwald ripening is considered the main factor of its instability. Nanoemulsion can be classified into two types; oil-in-water (o/w), which is the oil droplets are dispersed in continuous aqueous phase and water-in-oil (w/o), which is the water droplets are dispersed in continuous oil phase. It can be prepared by high- or low-energy processes. Several attractive advantages of nanoemulsions for application in personal care and cosmetics are good appearance and skin feel, effective delivery system for active ingredients, safety, enhanced actives solubility, and stability. The factors affecting droplet size of nanoemulsion were reported including type of oil and surfactant,[1] surfactant to oil ratio,[2] and preparation methods. Moreover, the main process parameters affecting the droplet size were the pressure, the temperature and the number of cycles.[3] Nanoemulsions are considered as thermodynamically unstable system, which pronounces that its stability is very important. The hydrophilic-lipophilic balance (HLB) is an index of solubilizing properties of surfactants, scale ranges between 0 and 20.[4] The surfactant systems with HLB values in range of 3–6 usually produce w/o nanoemulsion while the systems with HLB values in range of 8–16 tend to produce o/w nanoemulsion.[5] HLB plays an important role in the stabilization of nanoemulsions. The previous studies showed that the HLB value of the prepared emulsions could influence the emulsion stability.[6,7] In addition, the emulsions prepared with Span-Tween blends were more stable than that using single surfactant because the lipophilic hydrocarbon chain of Tween® could be located between Span® molecules at the interface, increased van der Waals attraction and resulted in the condensed interfacial film.[6] Moreover, it has been found that not only the HLB, but also the chemical structure of the surfactants affected the stability of the emulsion.[8] The study done by Hong *et al*.[8] reported that the emulsions with the mixture of Span® 80 and Tween® 80 showed greater stability than the emulsions with mixture of Span® 60 and Tween® 60. This could be explained by the fact that Span® 80 and Tween® 80 contain a double bond, resulting in more hydrophilic than a linear hydrocarbon chain which could allow better arrangement at the interfacial film of o/w emulsion. Soybean oil is one of the vegetable oils which have been used to develop nanoemulsions, because it contains fatty acids, such as linoleic acid and oleic acid, which are known as penetration enhancers.[9] Moreover, the previous clinical study showed that soybean oil provided the moisturizing effect to the skin by reducing transepidermal water loss.[10] The previous studies reported that o/w nanoemulsions using soybean oil in range of 8–12% exhibited good physical stability.[11-14] In addition, soybean oil nanoemulsions of 5-aminolevulinic acid[14] showed great enhancement of skin permeability when compared to the control solution. In this study, nanoemulsions were prepared using a high-energy process to produce a small droplet size. Various HLB values were obtained using the combinations of hydrophilic and lipophilic surfactant to achieve the required HLB. Tween® 80 and Span® 80 were selected according to their previously succeeds in stabilizing nanoemulsions with various oil types,[15-17] including soybean oil.[13] The objective of this work is to study the effect of the HLB values of non-ionic surfactants on physical property and physical stability of o/w soybean oil nanoemulsions.

MATERIALS AND METHODS

Materials

Soybean oil was purchased from Tip® vegetable oil company limited (Thailand). Tween® 80 (polyoxyethylene[18] sorbitan monooleate) was purchased from CRODA (Singapore). Span® 80 (sorbitan oleate) was purchased from NOF Corporation (Japan). Ultrapure water was used in this study.

Preparation of Nanoemulsions

The oil phase consisted of 10% w/w soybean oil and 5% w/w surfactant mixture (Tween® 80 and Span® 80). The HLB values were varied (i.e., HLB 4.3, HLB 8, HLB 9, HLB 10, and HLB 15) to obtain an optimum ratio of surfactant mixture that produced stable nanoemulsion. The emulsions were prepared by mixing the oil phase and the water phase through high-speed homogenizer at 5000 rpm for 5 min to obtain a coarse emulsion, then passing the coarse emulsion through the Microfluidizer LM20 (Microfluidics Inc., USA) at 100 MPa for four passes to obtain nanoemulsions and using outlet cooler to cool down nanoemulsions to 25°C.[11] The resulting nanoemulsions were left overnight at ambient temperature and protected from light to reach an equilibrium before characterization.

Type of Nanoemulsion

The nanoemulsions were diluted with ultrapure water or oil in 1:1 ratio to observe homogenization. If the water could be mixed with the sample, the nanoemulsion was classified as oil-in-water (o/w) but if the oil could be mixed with the sample, the nanoemulsion was classified as water-in-oil (w/o).

Determination of Size and Size Distribution

The average droplet size and size distribution (polydispersity index [PDI]) were measured by dynamic light scattering Zetasizer Nano ZS™ (Malvern Instruments, UK) with each measurement being the average of ten runs, each of 60 s duration. The nanoemulsions were diluted 1:100 with ultrapure water before the measurement.[14]

Physical Stability Studies

Preliminary physical stability screening by centrifugation

The nanoemulsions were centrifuged at 3500 rpm and 25°C for 30 min to observe phase separation.[19] Only the formulations with no phase separation were further evaluated.

Physical stability by heating-cooling cycles

The nanoemulsions were kept at 4°C for 48 h following by 45°C for 48 h as one cycle. The average droplet size and PDI of all samples were measured at the end of each cycle until six cycles.[20]

Physical stability at room temperature

The nanoemulsions were stored at room temperature and sampling for droplet size and PDI measurement every 4 days until 24 days. The Ostwald ripening rates were obtained from the slope of radius of droplet size^3 (r^3) versus time and referred from Equation 1.[18]

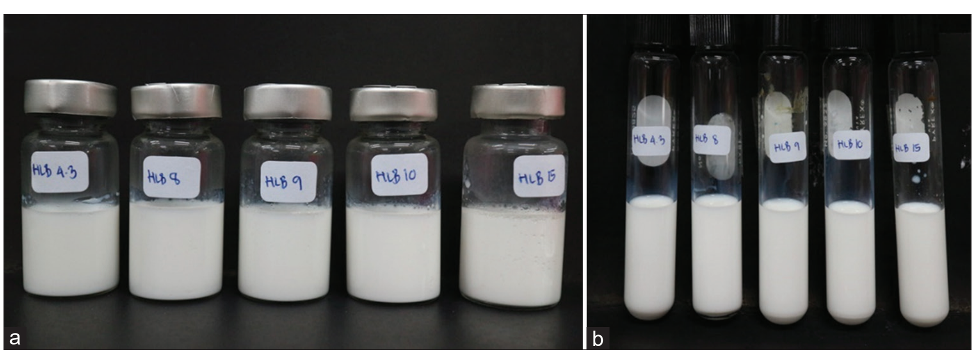

Figure 1: Appearance of the nanoemulsions before (a) and after centrifugation (b) with hydrophilic-lipophilic balance values of 4.3, 8, 9, 10, and 15 (from left to right)

Table 1: Droplet size, size distribution (PDI), and Ostwald ripening rates of the nanoemulsions (mean±SD, *n*=3)

Formulation	Tween® 80 (%w/w)	Span® 80 (%w/w)	Size (nm)	PDI	Rate (ω) (nm^3/h)
HLB 4.3	-	5.00	157.3±1.5	0.117±0.017	0.0013
HLB 8	1.73	3.27	169.4±1.1	0.081±0.015	0.0005
HLB 9	2.20	2.80	178.8±1.2	0.087±0.009	−0.0013
HLB 10	2.66	2.34	185.1±2.4	0.071±0.011	0.0015
HLB 15	5.00	-	228.6±1.1	0.092±0.019	−0.0056

PDI: Polydispersity index

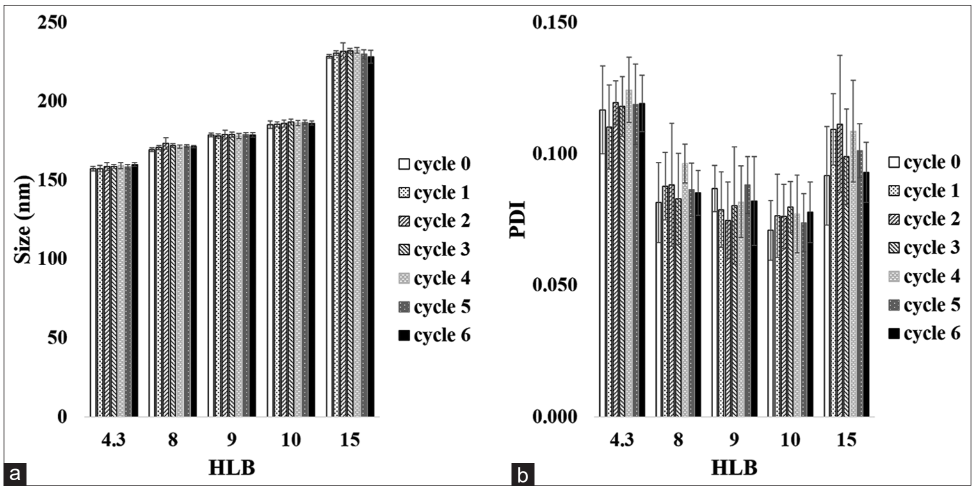

Figure 2: Droplet size (a) and polydispersity index value (b) of the nanoemulsions after heating-cooling cycles (mean ± SD, *n* = 3)

$$\omega = \frac{dr_N^{\ 3}}{dt} \qquad \text{Eq (1)}$$

where ω = Ostwald ripening rate
t = time (h)
r = droplet size of nanoemulsion in radius diameter (nm)

Data Analysis

All data were reported as mean ± standard deviation. Mean values were compared by one-way analysis of variance followed by Tukey's test for multiple comparisons. Differences were considered statistically significant when $P < 0.05$.

RESULTS AND DISCUSSION

Preparation of Nanoemulsions

Figure 1a showed that the appearance of all nanoemulsions was turbid liquids with the droplet size in range of 157.3–228.6 nm. No creaming was observed. All nanoemulsions were classified as o/w type because they could be mixed with water. Regarding to HLB 4.3, lipophilic surfactant Span® 80 also provided o/w type nanoemulsion because the formulations contained only 10% of an oil phase. However, the emulsions with a single surfactant used tend to be less stable in the long-term storage.[6] The mixtures of two or more surfactants were preferred. In this study, the mixtures of Tween® 80 and Span® 80 were selected for further studies and additional discussion also presents in the next section.

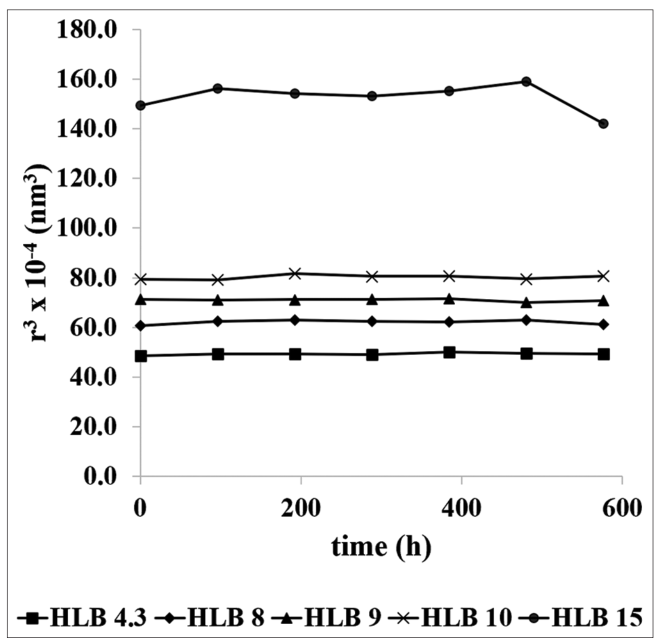

Figure 3: Ostwald ripening plot of the nanoemulsions with different hydrophilic-lipophilic balance values

Size and Size Distribution

Nanoemulsions prepared from single surfactant or mixed surfactants with different HLB values showed significant difference in the average droplet size ($P < 0.05$). A decrease in HLB value led to a decrease in droplet size. The result was in

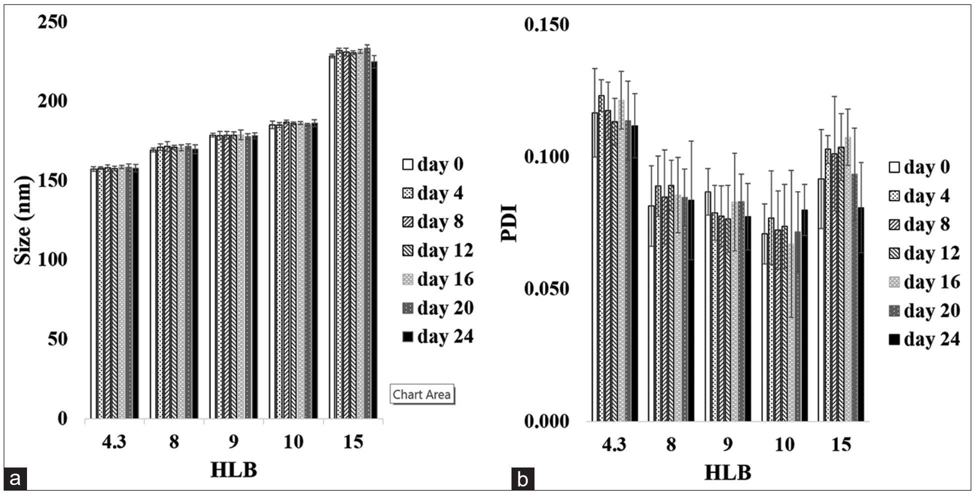

Figure 4: Droplet size (a) and polydispersity index value (b) of the nanoemulsions after storage at room temperature (mean ± SD, $n = 3$)

agreement with the previous study by Chong *et al*.[21] Despite the HLB 4.3 gave the smallest droplet size, it was unsuitable for use in o/w nanoemulsion. Surfactants with a range of HLB value in 8–18 are suitable to be used for o/w emulsion. Regarding to the stability study of the emulsions done by Takamura *et al*.[6] showed that the drainage rate of the emulsions using single surfactant was higher than that of mixed surfactants. The stable emulsions were formed using the combination of the hydrophilic surfactant (Tween®) and lipophilic surfactant (Span®). The hydrocarbon chain of Span® molecules was absorbed in the oil droplet where the sorbitan ring (hydrophilic portion) was in the aqueous phase. The hydrocarbon chain of Tween® molecules could be located in the oil droplet between the hydrocarbon chains of the Span®, and this could increase van der Waals force, resulting in strengthened interfacial film.[7] Thus, the optimum HLB is required for the nanoemulsions to protect the droplets against coalescence which not practically producing by any single surfactant. The mixed surfactant of the HLB 8 gave the smallest nanoemulsion droplet size among others of the mixed surfactants and smaller than Tween® 80. This result might be because the higher ratio of Span® 80 could cope with greater adsorption on smaller-sized oil droplet in nanoemulsions.[21] However, the result in the present study did not agree with the previous research that studied effect of HLB values on nanoemulsion size.[22,23] It might be due to the different concentrations and types of oil, surfactant, and the preparation method. All formulations gave narrow size distribution of droplets (PDI) [Table 1]. It indicated that these high-energy process parameters including pressure and number of cycles were efficient to produce soybean oil nanoemulsions with homogeneous dispersion of droplet size.

Physical Stability

Preliminary physical stability screening by centrifugation

All formulations passed the preliminary screening and showed no phase separation after centrifugation as shown in Figure 1b.

Physical stability by heating-cooling cycles

To investigate the thermal stability, the nanoemulsions were tested by heating-cooling cycles. The result showed that the average droplet sizes and PDI values of all formulations remained unchanged as shown in Figure 2.

Physical stability at room temperature

Similarly, the Ostwald ripening rates of all formulations which were obtained from the slope [Figure 3] were not statistically different and were almost zero [Table 1]. All nanoemulsions showed no significant change in droplet size and PDI as shown in Figure 4. The nanoemulsions stored at room temperature exhibited good physical stability.

CONCLUSION

The study showed that the o/w soybean oil nanoemulsions were successfully prepared through the Microfluidizer at 100 MPa for four passes. The HLB of surfactant system used in the preparation of nanoemulsions influenced the droplet size and size distribution. An increase in HLB value led to an increase in droplet size of nanoemulsions. However, all nanoemulsion formulations showed good physical stability after heating-cooling cycles and room temperature storage for at least 24 days. Nanoemulsions, a nanosized system, could efficiently enable active(s) movement into the skin because their small droplet sizes and remarkably high surface area could provide greater absorption into the stratum corneum than the large droplet size.[24,25] Although the formulation with Span® 80 presented the smallest droplet size, the formulation with HLB 8 which had the acceptable small droplet size would be preferred for the future development of cosmetics because it contains the mixed surfactant system which proposes to be more stable than the emulsions with single surfactant system due to more condensed interfacial film.

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