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Effects of surfactant mixture ratio and concentration on nanoemulsion physical stability

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Keywords: Nanoemulsions; Tween 20®; Transcutol®; Physical stability; Ostwald ripening rate

Objective: The aim of this study is to study the effects of surfactant mixture (smix) ratio and concentration on physical properties and physical stabilities of nanoemulsions.
Methods: Oil-in-water nanoemulsions were prepared by high-pressure homogenization method. Caprylic/capric triglycerides was used as oil phase. Surfactant mixtures between Tween 20® and Transcutol® were varied with different ratios and concentrations. Nanoemulsions were characterized for physical appearances, particle size and size distribution. Their physical stabilities were tested using centrifugation, heating-cooling cycle, and Ostwald ripening rate after storage at room temperature.
Results: The mean particle sizes of nanoemulsions were in the range of 88.3 to 227.2 nm with narrow size distribution (polydispersity index of 0.086-0.197). Smaller particle sizes were obtained with an increase in smix concentration. However, high Tween 20®, Transcutol®, or total smix concentration showed high Ostwald ripening rate and resulted in instable nanoemulsions when stored in accelerated conditions. F1 formulation containing the least amount of surfactant and only Tween 20® presented the lowest Ostwald ripening rate of 3.70×10^{-2} nm^3/h and was the most stable nanoemulsion.
Conclusion: Smix ratio and smix concentration showed influences on nanoemulsion physical properties and especially stabilities. Therefore, further works need to be done in order to develop more stable nanoemulsions.

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Introduction

Nanoemulsion is optically translucent to opaque flowable liquid due to its droplet size ranging between 20-200 nm[1]. It is kinetically stable and non-equilibrium system. In general, nanoemulsion consists of three main compositions namely oil, surfactant (and co-surfactant), and aqueous. Most cases, surfactants alone may insufficiently reduce the interfacial tension between oil and water to form small droplet[1]. The addition of short to medium chain alcohols as co-surfactant can also reduce more oil/water interfacial tension. They locate between hydrocarbon tails of surfactant, which could stabilize the interfacial layer of nanoemulsions. Hence, surfactant and co-surfactant are considered as important parts, which can influence nanoemulsion formation in terms of particle size and stability. Surfactant/co-surfactant mixture (smix) ratios and concentrations were studied in nanoemulsion formulations[2-4], which the high amount of surfactant(s) showed smaller in particle size, narrow size distribution and better stability. However, the effect of smix ratio on particle size and stability were not inevitable and depended on types of oil, surfactant/co-surfactant, and also their proportions. In this study, we prepared oil-in-water nanoemulsions of different smix ratios and smix concentrations using high-pressure homogenization method in order to observe their effects on particle size, size distribution and physical stabilities. Transcutol® was selected as a co-surfactant for further development of silymarin nanoemulsions. Not only Transcutol® helps in improving silymarin solubility[5] but also it is considered safe with low toxicity[6].

Methods

Preparation of nanoemulsions: Oil-in-water nanoemulsions were prepared by a modified high pressure homogenization method[7]. First, ultrapure water was added into the mixture of caprylic/capric triglycerides (CCT), Tween 20® and Transcutol®, and then the mixture was stirred on a magnetic stirrer for 30 minutes. Then, pre-emulsions were prepared using high speed homogenizer (D500, Wiggen Hauser) at speed of 22,000 rpm for 10 minutes. After that, they were further homogenized by high pressure homogenizer (Emulsiflex C5) at 1,000 bar for 6 cycles. All processes were performed at room temperature.

Characterization of nanoemulsions: The physical appearances of nanoemulsions were evaluated in terms of phase separation and creaming at 24 hours after preparation.

Particle size and size distribution: Particle size and size distribution were analyzed using dynamic light scattering (Nano ZS, Malvern) at a scattering angle of 173° using a 633 nm laser[3]. Nanoemulsions were diluted with ultrapure water (1:100, v/v) to avoid multiple scattering. Samples were measured after 10 seconds of equilibration at 25 °C.

Physical stability studies:

- Centrifugation: Nanoemulsions were centrifuged at 3,500 rpm for 30 minutes at 25 °C[8], and then were observed with respect to phase separation. Only those formulations without phase separation were selected for further studies.

- Heating-cooling cycle: Nanoemulsions were kept in the refrigerator at 4 °C for 48 hours and in the hot air oven at 45 °C for 48 hours per one cycle[9]. The test was carried out for six cycles. At the end of each cycle, measurements of particle size and size distribution were performed in triplicate and nanoemulsions were optically evaluated in terms of phase separation and creaming.

- Room temperature: Nanoemulsions were kept at 25 °C and their particle sizes were measured at the same time duration as heating-cooling cycle. At each time point, sample was diluted to 1:100 v/v with ultrapure water, which was proven not to affect the assessment of the Ostwald ripening rate (ω). The Ostwald ripening rate was obtained from the slope of r^3 versus time explained by Lifshitz-Slyozov-Wagner or LSW theory and referred from Equation 1[3].

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

where ω = Ostwald ripening rate; t = time (hour);
r = particle size of nanoemulsion in radius diameter (nm)

Results

Oil-in-water nanoemulsions were successfully prepared by high-pressure homogenization method using CCT as oil phase, ultrapure water as aqueous phase, Tween 20® as surfactant and Transcutol® as co-surfactant. Surfactant and co-surfactant mixture (smix) ratios were varied (i.e., 1:0, 1:0.14, 1:0.25, 1:0.36, 1:0.5, 1:1, 1:1.5, and 1:2) with different concentrations in the range of 10% to 30% smix. The physical appearances of freshly prepared nanoemulsions were opaque white liquid with low viscosity. There was no creaming or phase separation after 24 hours of preparation.

Particle size and size distribution: As shown in Table 1, the mean particle sizes (z-average) of nanoemulsions were in the range of 88.3 to 227.2 nm. The F12 formulation containing the most Transcutol® showed oversized particles (>200 nm) and was excluded for the further studies. All formulations had narrow size distribution and uniform size with very low values of polydispersity index (0.086-0.197).

Physical stability studies:

***-* Centrifugation:** Nanoemulsions were centrifuged which was accelerated their kinetic stability by force. Most nanoemulsions showed no change after centrifugation, except three formulations containing high amounts of Tween 20® (F7, F8, F9) showed phase separation with the upper layer of cream and the lower layer of cloudy water and were eliminated. Therefore, other nanoemulsions were further accelerated by temperature.

- Heating-cooling cycle: The heating-cooling cycle stability testing of nanoemulsions were determined by observing their physical appearances and also the growth of particle size at the end of each cycle. The results showed that means particle sizes of all increased after the first cycle of the test and phase separation occurred when the mean particle size exceeded 200 nm (data not shown). Nanoemulsion F1 that contains only Tween 20® without Transcutol® revealed the most stable formulation and passed through 5 cycles of heating-cooling (Table 1).

- Room temperature: Nanoemulsions were stored at room temperature and their particle sizes were measured at the same time point of heating-cooling cycle. The Ostwald ripening rates were calculated from the slopes of linear equation of graph plot (Figure 1), as shown in Table 1. The most stable nanoemulsion, F1, showed the lowest Ostwald ripening rate of 3.70×10^{-2} nm^3/h, while the F11 formulation containing high amount of Transcutol® as well as high smix concentration of 25% showed the highest Ostwald ripening rate of 87.20×10^{-2} nm^3/h.

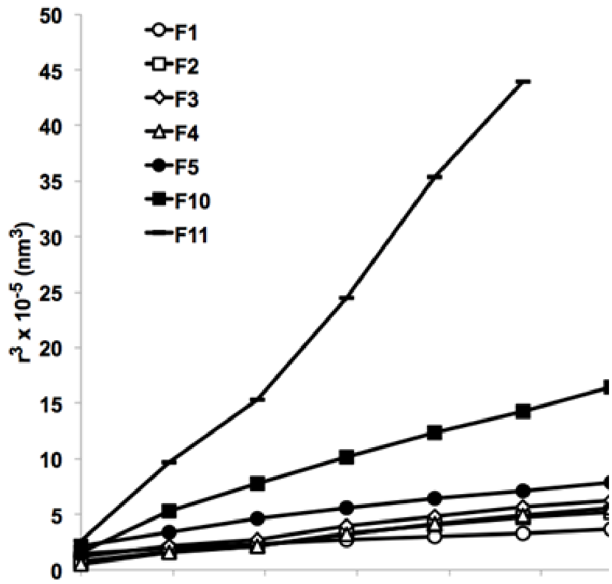

Figure 1. Ostwald ripening plot of nanoemulsions with different smix ratios and concentrations

Discussion

At a constant smix ratio of 1:0.5 (F5-F9), increasing the concentration of smix likely resulted in particle size reduction, except formulation F9. Among this smix ratio of 1:0.5 formulations, the higher smix concentration (>20%; F7, F8, F9 in Table 1) showed poorer stability in centrifugation study. The least smix concentration (F5) revealed more stable than other smix ratio of 1:0.5 formulations in heating-cooling cycle. This may imply that a small particle size did not always yield more stable nanoemulsion. Optimum smix concentration should take into consideration at specific smix ratio.
At a constant smix concentration of 15% (F2-F5), increasing the proportion of Transcutol® resulted in larger particle size. In heating-cooling cycle, F2 and F3 formulations containing 13% and 12% Tween 20®, respectively revealed less stable when compared with F4 and F5 (Table 1). In addition, the amount of Tween 20® was fixed at 10% where increasing in Transcutol® (F1, F5, F10, F11, and F12), larger particle sizes were obtained same as mentioned above.
Heating-cooling cycle showed the results in corresponding to Ostwald ripening rates, where the higher rate showed less stable formulation in heating-cooling cycle. There was an explanation about phase separation in heating-cooling cycle may be caused by an excess surfactant in the aqueous phase resulting in the enhancement of Ostwald ripening rate at room temperature[3]. An excess surfactant in aqueous phase may form micelles with oil and oil was transported from the internal phase to external phase then causing phase separation. Transcutol® seemed to have a negative effect on nanoemulsion stability, which may be caused by imperfect surfactant/co-surfactant film formation.

Conclusion

Smix ratio and concentration showed some influence on particle size of freshly prepared nanoemulsions. Both formulation parameters were very important to nanoemulsion stabilities. Further studies are required to obtain more stable nanoemulsions using the optimum smix ratio and concentration.

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Table 1. Mean particle size, size distribution and physical stabilities of nanoemulsions prepared with different smix ratios and concentrations

Formulation	Smix Ratio	Smix Concentration	Composition (% W/W)			Size (nm)	PdI[a]	Physical stability studies		$\omega \times 10^{-2}$ (nm^3/h)
			CCT	Tween 20®	Transcutol®			Cent.[b]	HC cycle[c]	
F1	1 : 0	10	10	10	-	113.8 ± 0.17	0.152 ± 0.008	nps	5 cycles	3.70
F2	1 : 0.14	15	10	13	2	96.0 ± 1.48	0.171 ± 0.014	nps	2 cycles	7.97
F3	1 : 0.25	15	10	12	3	90.8 ± 2.49	0.153 ± 0.007	nps	2 cycles	9.08
F4	1 : 0.36	15	10	11	4	106.0 ± 1.36	0.157 ± 0.011	nps	3 cycles	8.83
F5	1 : 0.5	15	10	10	5	119.8 ± 1.07	0.168 ± 0.006	nps	3 cycles	9.80
F6	1 : 0.5	18	10	12	6	91.2 ± 0.68	0.197 ± 0.006	nps	1 cycle	N/A
F7	1 : 0.5	21	10	14	7	89.6 ± 0.17	0.188 ± 0.110	ps	N/A	N/A
F8	1 : 0.5	24	10	16	8	88.3 ± 1.13	0.161 ± 0.009	ps	N/A	N/A
F9	1 : 0.5	30	10	20	10	102.2 ± 0.70	0.091 ± 0.012	ps	N/A	N/A
F10	1 : 1	20	10	10	10	118.5 ± 1.82	0.147 ± 0.002	nps	1 cycle	25.10
F11	1 : 1.5	25	10	10	15	148.7 ± 0.59	0.086 ± 0.014	nps	1 cycle	87.20
F12	1 : 2	30	10	10	20	227.2 ± 0.70	0.109 ± 0.015	N/A	N/A	N/A

[a] PdI, polydispersity index
[b] Cent., centrifugation, nps : no phase separation, ps : phase separation
[c] HC cycle, heating-cooling cycle, reported as the number of stable cycles
ω, Ostwald ripening rate
N/A, Not available
Mean ± SD, n = 3