Colloidal Stability of Emulsifier-free Triolein-in-Water Emulsions: Effects of Temperature

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Abstract: Herein, we report the colloidal stability of emulsifier-free (EF-) triolein-in-water (TO/W) emulsions prepared by mixing TO and water using a high-powered bath-type ultrasonicator (HPBath-US; 28 kHz, 300 W) in the absence of emulsifiers such as surfactants. In particular, the effect of the temperature (15–60°C) on the colloidal stability of EF-TO/W emulsions was examined because this is important for the practical application of EF-TO/W emulsions, for example, in foods, pharmaceuticals, and cosmetics. We found that the colloidal stability of the EF-TO/W emulsions decreased with increase in the temperature from 15 to 25°C, whereas it increased with increase in temperature from 25 to 40°C, and the high colloidal stability of the EF-TO/W emulsions was maintained above 40°C. The reduction in the colloidal stability of EF-TO/W emulsions between 15 and 25°C is likely a result of the TO droplets formed by thermal motion, as well as enhanced Ostwald ripening at higher temperatures. On the other hand, the increase in the colloidal stability of the EF-TO/W emulsions from 25 to 40°C and their high colloidal stability above 40°C is attributed to the reduction in the interfacial tension between TO and water at higher temperatures. This decrease in the interfacial tension between TO and water with temperature increase is related to the transformation of short-range ordered domains (clusters) of TO molecules in the liquid state, which increases the colloidal stability of the EF-TO/W emulsions.

Key words: emulsifier-free triolein-in-water emulsion, colloidal stability, temperature, interfacial free energy, short-range ordered domain

1 Introduction

Oil-in-water (O/W) and water-in-oil (W/O) emulsions are used in various products and manufacturing processes in a broad range of industries, including the food, cosmetics, pharmaceutical, agrochemical, paint, and oil industries^{1–3)}. This popularity is a result of the unique properties of emulsions afforded by the liquid droplets (dispersed phase), continuous phase, and interface between oil and water phases. Typically, the preparation and stabilization of emulsions require emulsifiers, such as surfactants or amphiphilic polymers. However, in recent years, emulsifier-free (EF-) emulsion products, which are prepared in the absence of any emulsifiers, have become in-demand because of the rise in green products and green manufacturing^{4–25)}. For example, in the food industry in the last few years, the “clean label” concept has become popular because consumers increasingly prefer the use of natural, safe, and

sustainable materials, and as a result, many manufacturers have focused on the development of EF-products^{26–28)}. Therefore, it is important to understand the fundamental nature of EF-emulsions: for example, the effect of the oil properties and the effects of the preparation and storage temperature on the colloidal stability of EF-O/W and EF-W/O emulsions. Therefore, we have previously investigated the effects of the oil properties on the colloidal stability of EF-O/W and EF-W/O emulsions to evaluate the fundamentals of the colloidal stabilization of emulsions^{4–16)}.

In contrast, in this work, the effect of the preparation and storage temperatures on the colloidal stability of EF-triolein-in-water (EF-TO/W) emulsions was examined because triolein (TO) is a typical triacylglycerol (TG) that is frequently used in foods, pharmaceuticals, and cosmetic products, and temperature is one of the most important parameters affecting the manufacture and storage of emul-

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sion-based products.

2 Experimental

2.1 Preparation of EF-TO/W emulsions

EF-TO/W emulsions were prepared by mixing 0.1 mL of TO ($C_{57}H_{104}O_6$, >99%, Merck KGaA) and 25 mL of ultrapure water (18.2 M Ω cm at 25°C, Millipore-filtered water) using a high-powered bath-type ultrasonicator (HPBath-US; 28 kHz, 300 W) for 5 min at 15, 20, 25, 30, 35, 40, 50, and 60°C.

2.2 Characterization of colloidal stability of EF-TO/W emulsions

The colloidal stabilities of the prepared EF-TO/W emulsions were characterized by monitoring the turbidity of the EF-TO/W emulsions with time from the moment of preparation. For this, the prepared EF-TO/W emulsions were stored at 15, 20, 25, 30, 35, 40, 50, and 60°C. The turbidity of the EF-TO/W emulsion was obtained from the difference between the transmittances of the continuous phase (water phase) and EF-TO/W emulsion at a wavelength of 700 nm, as measured with a UV-visible spectrophotometer (V-630, JASCO). The change in the turbidity of the EF-TO/W emulsion with elapsed time is caused by the creaming (floating) of TO droplets in the EF-TO/W emulsion because the light used for the turbidity measurement passes through a window positioned 5.0 mm from the bottom of the glass cuvette, i.e., far from the location of the separated oil.

2.3 Characterization of the diameter and zeta potential of TO droplets in EF-TO/W emulsions, densities of water and TO, and interfacial tension between TO and water

The diameter and zeta potential of TO droplets in the EF-TO/W emulsions prepared at 15, 20, 25, 30, 35, 40, 50, and 60°C were measured at the same temperatures as the preparation temperatures using laser diffraction (LA-950, HORIBA) and electrophoretic light scattering (Zetasizer Nano, Malvern), respectively. The densities of water and TO at 15, 20, 25, 30, 35, 40, 50, and 60°C were measured with a standard specific gravity meter (19-4, AS ONE). The interfacial tension (γ) values between TO and water at 15, 20, 25, 30, 35, 40, 50, and 60°C were measured using the Wilhelmy method (CBVP-A3, Kyowa Interface Science Co., Ltd.).

3 Results and Discussion

The white color of the EF-TO/W emulsions prepared and stored at 15, 20, and 35°C gradually became light white

Fig. 1 EF-TO/W emulsions observed with elapsed time after preparation at 15, 20, 25, 30, 35, 40, 50 and 60°C.

over 30 d after preparation (see Fig. 1). In the case of the EF-TO/W emulsions prepared and stored at 25 and 30°C, the creaming of oil droplets was observed over 30 d after preparation, in which the white color of the EF-TO/W emulsions moved to the upper region and the bottom of the vessel became light white (see Fig. 1). No significant changes over 30 days after preparation were observed for the EF-TO/W emulsions prepared and stored between 40 and 60°C (see Fig. 1). The turbidities of the EF-TO/W emulsions prepared and stored at 15, 20, and 35°C slightly decreased with time after preparation (see plot markers ○, ●, and △ in Fig. 2). The turbidities of the EF-TO/W emulsions prepared and stored at 25 and 30°C decreased with time after preparation (see plot markers □ and ■ in Fig. 2). On the other hand, the turbidities of the EF-TO/W emulsions prepared and stored between 40 and 60°C showed negligible changes over 30 d after preparation (see plot markers ▲, ▽ and ▼ in Fig. 2). The turbidity values of the EF-TO/W emulsions measured 20 d after preparation and plotted as a function of preparation and storage temperature decreased and increased as the temperature was increased from 15 to 25°C and from 25 to 40°C, respectively, but plateaued above 40°C (see Fig. 3). That is, the colloidal stabilities of the EF-TO/W emulsions decreased in the lower temperature range and increased in the mid-temperature range. The decrease in the colloidal stability

Fig. 2 Turbidity of EF-TO/W emulsions recorded with elapsed time after preparation at (○) 15, (●) 20, (□) 25, (■) 30, (△) 35, (▲) 40, (▽) 50 and (▼) 60°C.

Fig. 3 (●) Turbidity of EF-TO/W emulsions measured at 20 d after preparation plotted as a function of temperature. Plot represents average turbidity of EF-TO/W emulsions measured at 20 d after preparation, and error bar represents the minimum and maximum of turbidity of EF-TO/W emulsions in each measurement (3 times) at 20 d after preparation.

of the EF-TO/W emulsions between 15 and 25°C can be attributed to the enhanced merging of TO droplets, as well as increased Ostwald ripening, with increasing temperature, which is common temperature-dependent behavior of O/W emulsions. For comparison, Fig. S1 shows the turbidity of EF-dodecane-in-water (DD/W) emulsion and EF-hexadecane-in-water (HD/W) emulsions measured 5 d after preparation and plotted as a function of preparation and storage temperatures. As shown, over 5 d, the turbidity

values of the EF-DD/W and EF-HD/W emulsions decreased with increase in temperature from 15 to 60°C. This result indicates that the colloidal stability of the EF-TO/W emulsions on increase in temperature from 25 to 40°C and the high colloidal stability of EF-TO/W emulsions above 40°C is a unique feature of O/W emulsions.

First, the effect of creaming on the colloidal stability of EF-TO/W emulsions was examined to evaluate the mechanism of the increase in the colloidal stability of EF-TO/W emulsions on increase in temperature from 25 to 40°C. Generally, O/W emulsions are demulsified through creaming, which involves the floating of oil droplets in O/W emulsions or the sedimentation of water droplets in W/O emulsions. The creaming of O/W emulsions is expressed by Stokes' law (Eq. 1)²⁹⁾.

$$u = \frac{2r^2(\rho_0 - \rho)g}{9\eta} \quad (1)$$

Here, u (m/s) is the floating velocity of oil droplets in an O/W emulsion, r (m) is the radius of the oil droplets, g (m/s²) is gravitational acceleration, ρ_0 (kg/m³) is the mass density of water (continuous phase), ρ (kg/m³) is the mass density of oil (dispersed phase), and η (Pa s = kg/m·s) is the viscosity of water (continuous phase). The median diameters of the TO droplets in the EF-TO/W emulsions immediately after preparation between 40 and 60°C were slightly smaller than those prepared from 15 to 35°C (see Fig. 4a). On the other hand, the changes in the TO droplet size distribution and median diameter in the EF-TO/W emulsions over 7 d after preparation at temperatures between 15 and 60°C were similar (see Figs. S2 and S3). Therefore, the smaller median diameters of the TO droplets in the EF-TO/W emulsion immediately after preparation at 40–60°C compared to those prepared at 15–35°C did not affect the increase in the colloidal stability of the EF-TO/W emulsions on storage at temperatures from 25 to 40°C or the high colloidal stability of the EF-TO/W emulsion above 40°C. In addition, the density difference between water and TO remained unchanged with increase in storage temperature (see Fig. 4b). That is, the increase in the colloidal stability of the EF-TO/W emulsion with increase in storage temperature from 25 to 40°C and high colloidal stability of EF-TO/W emulsion above 40°C cannot be attributed to changes in the density of TO with temperature.

Next, the effect of the repulsive electrostatic interactions between TO droplets (i.e., the zeta potential of the oil droplets) on the colloidal stability of the EF-TO/W emulsions was examined to elucidate the mechanism of the increase in the colloidal stability of the EF-TO/W emulsions at storage temperatures between 25 and 40°C. The repulsive electrostatic interactions between oil droplets in O/W emulsions can be explained by Derjaguin–Landau–Verwey–Overbeek (DLVO) theory^{30–34)}. This theory postulates that the colloidal stability of O/W emulsions is controlled by the

Fig. 4 (a) Median diameter of TO droplets in EF-TO/W emulsions right after preparation, (b) Density difference between water and TO ($\rho_0 - \rho$), (c) zeta-potential of TO droplets in EF-TO/W emulsions right after preparation and (d) interfacial tension between TO and water at 15, 20, 25, 30, 35, 40, 50 and 60°C. Plot represents average value, and error bar represents the minimum and maximum in each measurement (3 times).

balance of attractive (V_A) and repulsive (V_R , see Eqs. 2 and 3) interactions between oil droplets in O/W emulsions. In particular, the repulsive interaction (V_R) between oil droplets in O/W emulsions is the dominant factor controlling the colloidal stability of O/W emulsions.

$$V_R = \frac{\epsilon r \zeta^2}{2} e^{-\kappa H} \quad (2)$$

$$\kappa = \sqrt{\frac{8\pi J F^2}{1000 \epsilon R T}} \quad (3)$$

Here, r (m) is the radius of the oil droplets, ϵ (F m⁻¹) is the dielectric constant of the oil (dispersed phase), ζ (V) is the zeta potential of the oil droplets, H (m) is the distance between the oil droplets, F (C mol⁻¹) is the Faraday constant, J (mol m⁻³) is the ionic strength, R (J mol⁻¹ K⁻¹) is the gas constant, and T (K) is the absolute temperature. κ (m⁻¹) is the Debye-Hückel parameter (Eq. 3), and the reciprocal of the Debye-Hückel parameter (κ^{-1}) represents the screening length of the electrical double-layer^{35, 36}. Therefore, the stability of the O/W emulsion increases with increase in the repulsive interaction (V_R) between oil droplets. Crucially, the O/W interface acquires a negative charge

even in the absence of emulsifiers such as surfactants³⁷. Therefore, the oil droplets in EF-O/W emulsions typically have a negative surface potential (zeta potential) ranging from 30 to 60 mV^{7, 9-11, 17}. The TO droplets in the EF-TO/W emulsion also have a negative zeta potential, ranging from 30 to 60 mV (see Fig. 4c). This surface charge of the oil droplets in the EF-O/W emulsion originates from the adsorption of hydroxide ions (OH⁻) in water onto the surfaces of the oil droplets, as confirmed by measurements of the change in the surface charge of oil droplets in EF-O/W emulsions as a function of pH³⁸⁻⁴⁰. The measured zeta potential of the TO droplets in the EF-TO/W emulsions gradually decreased with increase in temperature (see Fig. 4c), whereas the colloidal stability of the EF-TO/W emulsions increased with temperature from 25 to 40°C and was maintained above 40°C. Thus, the repulsive electrostatic interactions between TO droplets in the EF-TO/W emulsion is not the origin of the increase in their colloidal stability on increase in storage temperature between 25 or 40°C and their high colloidal stability above 40°C.

Finally, the mechanism of the increase in the colloidal

stability of the EF-TO/W emulsions between 25 to 40°C was examined in terms of the interfacial free energy (G), that is, the interfacial tension (γ) between TO and water. Because emulsions are a transient mixture of two immiscible liquids such as oil and water, they have high free energies (that is, interfacial free energies) (G) at the O/W interface¹⁻³. G is given by Eq. 4.

$$G = \gamma A \quad (4)$$

Here, γ (mN m⁻¹) is the interfacial tension between oil and water, and A (m²) is the area of the O/W interface. Emulsions are typically prepared using emulsifiers, such as surfactants, because these compounds decrease the value of γ between the oil and water phases, resulting in a decrease in G . As shown in Fig. 4d, the value of γ between TO and water dramatically decreases as the temperature is increased from 35 to 40°C. In contrast, the values of γ for dodecane and water and for hexadecane and water do not change significantly between 15 and 60°C (see Fig. S4), suggesting that the colloidal stability of the EF-TO/W emulsions increases with increase in temperature from 25 to 40°C and that the high colloidal stability at 40°C is a result of the decrease in γ and, thus, G between TO and water. The decrease in γ between TO and water between 35 and 40°C can be attributed to the structural transformation of short-range ordered domains (clusters) of TO molecules in the liquid state. Generally, short-range ordered domains (clusters) of TGs exist even in their liquid states, and the structures of these clusters change with temperature⁴¹⁻⁴⁶. For example, deuterated trilaurin forms a nematic-like cluster structure in the liquid state⁴¹. In addition, TGs form a labile paralamellae-like (smectic phase) structure rather than a nematic-like structure in the liquid state⁴²⁻⁴⁵. Moreover, Y-shaped TGs having three alkyl chains spread at 120° can form a cylindrical symmetrically overlapping "discotic phase"⁴⁶. This suggests that the transformation of the clusters of TO molecules in the liquid state of TO around 35–40°C results in a decrease in γ between TO and water on increasing the temperature and, thus, an increase in the colloidal stability of the EF-TO/W emulsions above 40°C.

Next, we discuss the colloidal stability of the EF-TO/W emulsions at temperatures from 25 to 40°C and their high colloidal stability above 40°C with respect to Ostwald ripening, that is, the process by which oil molecules diffuse from smaller droplets to larger droplets in water (as defined by Kelvin's law). As shown by Lifshitz-Slyozov-Wagner (LSW) theory (Eq. 5)^{47, 48}, an increase in temperature should promote Ostwald ripening because of the increase in the solubility of oil in water and the resulting demulsification of the EF-TO/W emulsion.

$$\omega_{\text{Ostwald}} = \frac{dr^3}{dt} = \frac{8Dc_{\infty}\gamma V_m^2}{9RT} \quad (5)$$

Here, ω_{Ostwald} ($= dr^3/dt$) (m³ s⁻¹) is the growth rate of the

oil droplets, r (m) is the radius of the oil droplets, t (s) is the time, D (m² s⁻¹) is the diffusion constant of oil molecules in water, c_{∞} (mol m⁻³) is the solubility of oil in water, γ (N m⁻¹) is the interfacial tension between oil and water, V_m (m³ mol⁻¹) is the molar volume of the oil, R (J mol⁻¹ K⁻¹) is the gas constant, and T (K) is the absolute temperature. As mentioned, Ostwald ripening between TO droplets in EF-TO/W emulsions should be favored by an increase in temperature because the solubility of TO in water increases with increasing temperature. As a result, the colloidal stability of EF-TO/W emulsions should decrease with increase in temperature. However, we observed the opposite trend; that is, an increase in colloidal stability at temperatures higher than 40°C. This indicates that the increase in the colloidal stability of the EF-TO/W emulsions as the temperature increased from 25 to 40°C and their high colloidal stability above 40°C are not attributed to Ostwald ripening. Furthermore, the changes in the size distribution of TO droplets in the EF-TO/W emulsions prepared at 15, 20, 25, 30, 35, 40, 50, and 60°C with time showed no significant changes over 7 d after preparation (see Fig. S2). The median diameters of the TO droplets in the EF-TO/W emulsions prepared at 15, 20, 25, 30, 35, 40, 50, and 60°C decreased from approximately 2.2 to 1.3 μ m over 7 d from preparation (see Fig. S3). The decrease in the median diameter of the TO droplets in the EF-TO/W emulsions with time after preparation is likely due to the floating of larger TO droplets. The trend in the decrease in the median diameter of TO droplets in the EF-TO/W emulsions is similar between 15 and 60°C (see Fig. S3). These results also indicate that the increase in the colloidal stability of the EF-TO/W emulsions with increase in temperature from 25 to 40°C and high colloidal stability of EF-TO/W emulsions above 40°C are not attributed to Ostwald ripening.

4 Conclusions

The colloidal stability of emulsifier-free triolein-in-water (EF-TO/W) emulsions decreases with increase in temperature from 15 to 25°C, whereas the colloidal stability of these emulsions increases as the preparation and storage temperatures increase from 25 to 40°C. The increase in the colloidal stability of the EF-TO/W emulsions as the preparation and storage temperatures increased from 25 to 40°C likely results from the decrease in interfacial tension (γ) between TO and water resulting from the transformation of short-range ordered domains (clusters) of TO molecules in the liquid state of TO around 35–40°C. This result suggests that the internal state of the oil droplets in O/W emulsions affects the interfacial state, and, simultaneously, the interfacial state affects the colloidal stability of the emulsion.

Supporting Information

This material is available free of charge via the Internet at doi: 10.5650/jos.ess21234

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