Effect of Addition and Molecular Size of Triglyceride Oils on Phase Behavior and Surfactant Self-Assemblies

Kenji ARAMAKI* and Hossain Md. KHALID

*Graduate School of Environment and Information Sciences, Yokohama National University
(Tokiwadai 79-7, Hodogaya-ku, Yokohama 240-8501)*

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Abstract: The cloud temperature of $C_{12}EO_8$ aqueous solution decreases by solubilizing triglyceride oils (1,2,3-trihexanoylglycerol (THG), 1,2,3-trioctanoylglycerol (TOG) and 1,2,3-tridecanoylglycerol (TDG)). The reduction of cloud temperature is larger with the oil having shorter alkyl chains. In the water/ $C_{12}EO_8$ system, a micellar, a hexagonal, a bicontinuous cubic and a lamellar phases are successively formed with increasing surfactant concentration at 25°C. A discontinuous cubic phase is formed by adding TOG to the aqueous $C_{12}EO_8$ system. SAXS measurement was performed on the discontinuous cubic, hexagonal and lamellar phases and molecular parameters such as effective cross sectional area per surfactant molecule, radius of micelle for the discontinuous cubic and the hexagonal phases, half-thickness of bilayer of the lamellar phase were obtained. The changes in the molecular parameters by increasing TOG content suggest that most of the oil molecules are solubilized in the core of surfactant aggregates. Thermal stability of the discontinuous cubic phase increases. The amount of solubilized oil in the discontinuous cubic phase increases by increasing temperature and decreasing oil molecular weight.

Key words: triglyceride oil, oil solubilization, cubic phase, phase behavior, small angle X-ray scattering

1 Introduction

Triglyceride oil (1,2,3-trialkanolyglycerol) is a main ingredient of food oils. Many food products are in a form of emulsion that mainly contains water, oil and emulsifier (normally surfactant). Emulsions can be categorized in two types, oil-in-water (O/W) or water-in-oil (W/O), which normally depends on the hydrophilic-lipophile balance (HLB) of surfactant used. However the emulsion type is also changed by the property of oil (1) even if the surfactant HLB is fixed.

Emulsions are normally formulated by shaking a sample of a multi-phase equilibrium, which is normally micellar phase and excess oil phase, or reverse micellar

phase and excess water phase. It is also possible to formulate emulsions in another multi-phase sample containing liquid crystal. It is reported that stable O/W or W/O emulsions can be formulated with utilizing lamellar or reverse hexagonal phase (2,3). Since dispersed phase is covered by liquid crystals, stability of the obtained emulsion is high. Kunieda *et al.* (4,5) reported that the emulsion formulated from the two-phase equilibrium of a discontinuous cubic phase and an oil phase is highly viscous and transparent due to the high viscosity of the continuous phase being the cubic phase and the contrast matching of refractive indices of the continuous phase (cubic phase) and the dispersed phase (oil). They also reported the gel-emulsion in which water

*Correspondence to: Kenji ARAMAKI, Graduate School of Environment and Information Sciences, Yokohama National University, Tokiwadai 79-7, Hodogaya-ku, Yokohama 240-8501
E-mail: aramakik@ynu.ac.jp

droplets are dispersed in a matrix of a reverse-discontinuous cubic phase (6).

Cubic phase is a gel-like phase with high viscosity and transparency. It is categorized in four types, discontinuous type, bicontinuous type, reverse bicontinuous type and reverse discontinuous type. Discontinuous and reverse-discontinuous-cubic phase contain spherical micelles with positive and negative curvature, respectively. The cubic-phase-stabilized emulsion mentioned in the previous paragraph has a continuous phase being the discontinuous or a reverse discontinuous cubic phase. The discontinuous-type cubic phase is normally formed between a micellar phase and a hexagonal phase in a phase diagram as a function of concentration of surfactant. Since spherical micelles have to maintain their structure at high surfactant concentration to form a discontinuous cubic phase, it is normally formed in aqueous surfactant systems with very hydrophilic non-ionic surfactants, e.g. poly(oxyethylene)-type surfactant (7), poly(oxyethylene)-poly(oxypropylene)-poly(oxyethylene) triblock copolymer (8), polyglycerol ester (9), sucrose ester (10). However adding oil to a micellar phase or a hexagonal phase in an aqueous surfactant system often induces a formation of a discontinuous cubic phase (5,11-13) since a structural transition of rod-like or elongated micelles to spherical micelles often takes place by the solubilization of oil (14). The amount of adding oil needed for the formation of discontinuous cubic phase increases with decreasing molecular size of hydrocarbons and decreasing surfactant hydrophilicity (12).

If the oil molecules are solubilized in the core of the self-assemblies, a positive surfactant layer curvature increases (1) or a zero curvature is unchanged (11). On the other hand if the oil molecules penetrate into a surfactant palisade layer, a positive curvature becomes less positive or even it is turned into a negative curvature (1). Note that a positive and a negative curvature of surfactant layer indicate a surfactant layer that is convex toward water and oil, respectively. The curvature of normal-type micelle or hexagonal phase tends to be less positive by the solubilization of oils with penetration tendency and to be more positive by the solubilization of oils with less-penetration tendency (1,11). Hence the phase transition from a micellar phase or a hexagonal phase to a discontinuous cubic phase takes place in the case of adding oils having less penetration tendency (13). In previous reports (1,11), hydrocarbons or aro-

matic oils are mainly used to discuss the relation between the solubilization site of oils and the structural change of self-assemblies. However there are few reports for triglyceride oils in spite of the importance of practical applications.

In this paper we report the phase behavior of water/poly(oxyethylene)-type nonionic surfactant/triglyceride oil systems to evaluate the effect of addition and molecular size of triglyceride oils on the structure of surfactant self-assemblies.

2 Experimental

2.1 Materials

Homogenous octa(oxyethylene) dodecyl ether ($C_{12}EO_8$) was purchased from Nikko Chemicals. 1,2,3-trioctanoylglycerol (tricaprylin, TOG) was purchased from Sigma-Aldrich Chemicals. 1,2,3-trihexanoylglycerol (tricaproin, THG) and 1,2,3-tridecanoylglycerol (tricaprin, TDG) were purchased from Tokyo Kasei Kogyo. These chemicals were used as received. Millipore filtered water was used to prepare the samples. Densities of $C_{12}EO_8$ (1.01 g cm^{-3}) and TOG (0.950 g cm^{-3}) were measured by the oscillating U-tube density meter (Anton Paar, DSA-5000).

2.2 Determination of Phase Diagrams

The samples were prepared individually by weighing the appropriate amounts of the components in glass ampoules. The ampoules were flame-sealed immediately and the sample mixtures were homogenized using a vortex mixer. Viscous samples were homogenized further by repeated centrifugation in both directions through narrow constriction. The ampoules were kept in a temperature-controlled bath at 25°C for several minutes or days to bring the system at equilibrium. Phase identification was done by direct visual inspection and observing the samples through cross polarizers.

2.3 Small-Angle X-ray Scattering (SAXS)

SAXS measurements were performed on a small-angle scattering camera equipped with rotating anode and a CCD 2-D detector (Rigaku, NanoViewer). Ni-filtered $\text{Cu-K}\alpha$ radiation ($\lambda = 1.541 \text{ \AA}$) was used. The applied voltage and filament current were 40 kV and 20 mA, respectively. The samples of liquid crystals were filled in a hole of an iron plate and covered by plastic (Mylar) films. Sample thickness was kept at 1 mm. The

interlayer spacing, d , of the liquid crystals was obtained from the first Bragg peak.

Radius of lipophilic core of spherical micelle, r_i , and a cross sectional area per surfactant molecule at the hydrophobic interface, a_s , for a discontinuous cubic phase are evaluated by the following eqs. (1) and (2).

$$r_i = d \cdot \left(\frac{3}{4\pi n_c} \cdot (\phi_L + \phi_o) \right)^{1/3} \cdot C \quad (1)$$

$$a_s = \frac{3v_L}{r_i} \cdot \frac{\phi_L + \phi_o}{\phi_L} \quad (2)$$

ϕ_L and ϕ_o are the volume fractions of lipophilic part of surfactant and oil in a system, respectively. v_L is the volume of lipophilic part of surfactant molecule, which is obtained by a summation of methyl and methylene unit volume (7). n_c is number of micelles in a unit cell of cubic phase. C is a constant. For a simple cubic structure, $n_c = 1$ and $C = 1$; a body-centered cubic structure, $n_c = 2$ and $C = \sqrt{2}$; for a face-centered cubic structure, $n_c = 4$ and $C = \sqrt{3}$.

Radius of lipophilic core of cylindrical micelle, r_H , and a_s for a hexagonal liquid crystalline phase are evaluated by the following eqs. (3) and (4).

$$r_H = \left\{ \frac{2}{\sqrt{3}\pi} (\phi_L + \phi_o) \right\}^{\frac{1}{2}} \cdot d \quad (3)$$

$$a_s = \frac{2v_L}{r_H} \cdot \frac{\phi_L + \phi_o}{\phi_L} \quad (4)$$

Half thickness of lipophilic part in a bilayer, d_L , and a_s , for a lamellar liquid crystalline phase are evaluated by the following eqs. (5) and (6).

$$d_L = \frac{d}{2} (\phi_L + \phi_o) \quad (5)$$

$$a_s = \frac{v_L}{d_L} \cdot \frac{\phi_L + \phi_o}{\phi_L} \quad (6)$$

3 Results and Discussion

3.1 Effect of Solubilization of Oil on Cloud Temperature

Cloud temperatures were measured by visual inspection for 2 wt% of aqueous solutions of $C_{12}EO_8$ with solubilized triglyceride oils. In **Fig. 1**, cloud temperatures are plotted against the oil content.

Cloud temperature of the aqueous $C_{12}EO_8$ solution

without oil is 78°C. Cloud temperature decreases by adding 0.25 wt% of triglyceride oils and it is almost unchanged with more addition of oil. Comparing three triglyceride oils, the largest decrease of cloud temperature at the early stage of solubilization of oil is for the system with THG. To compare with other oils, we also show the results from a literature (13). Saturated hydrocarbons which tend to be solubilized in the core of micelles does not affect cloud temperature so much whereas SLIP, m-xylene and 1-dodecanol which tend to penetrate into surfactant palisade layer decrease cloud temperature very much. Present three triglyceride oils show similar trend as for the saturated hydrocarbons, suggesting that these triglyceride oils tend to be solubilized in the core of micelles. Among the system with saturated hydrocarbons, cloud temperature is lower with a hydrocarbon having smaller molecular weight. The cloud temperature of the system with these triglyceride oils is also the highest for TDG having the largest molecular weight and the lowest for THG having the smallest molecular weight among three triglyceride oils.

Fig. 1 Cloud Temperatures of 2 wt% Aqueous Solution of $C_{12}EO_8$ with Solubilized Triglyceride Oils are Plotted against Oil Content. Cloud temperatures with other oils taken from a literature (13) are also plotted as a reference. SLIP indicates sarcosinate-lauroyl isopropyl.

3.2 Phase Behavior in a Water/ $C_{12}EO_8$ /TOG System

A phase diagram in the water/ $C_{12}EO_8$ /TOG system at 25°C is constructed and it is shown in **Fig. 2**.

On the water- $C_{12}EO_8$ axis, a micellar (W_m), a hexagonal (H_1), a bicontinuous cubic (V_1) and a lamellar (L_α) phase are successively formed with the increase in the surfactant concentration. As observed in various hydrophilic nonionic and ionic surfactant systems (15) the surfactant layer curvature of the binary water/ $C_{12}EO_8$ system is changed from highly positive to zero curvature. Note that a positive curvature is defined as a curvature which is convex toward water. When TOG is added to the aqueous micellar solution of $C_{12}EO_8$, it is solubilized in the micelle. However the amount of oil solubilized in the micellar solution is not very large, i.e. solubilized oil is less than 1 wt% at 10 wt% surfactant in water, less than 2 wt% at 20 wt% surfactant in water. Since $C_{12}EO_8$ is rather hydrophilic surfactant and its spontaneous curvature is large, it is not allowed forming large spherical micelles containing a

Fig. 2 Phase Diagram of Water/ $C_{12}EO_8$ /TOG System at 25°C. W_m , I_1 , H_1 , V_1 , L_α , O_m and S indicate a micellar phase, a discontinuous cubic phase, a hexagonal phase, a bicontinuous cubic phase, a lamellar phase, a reverse micellar phase and a solid phase, respectively. $W_m + O$, $I_1 + O$ and $H_1 + O$ indicate a two-phase equilibrium of W_m , I_1 and H_1 with an excess oil phase, respectively. The I_1 phase is formed as an island, which is separated from the water-surfactant axis.

large amount of solubilized oil. Oil-solubilization capacity in micelles and liquid crystals increases with the surfactant concentration since the curvature of surfactant layer becomes less positive and it allows forming large surfactant aggregates containing a large amount of oil.

If we see the change in self-assembled structures by adding TOG, H_1 - I_1 transition on the left hand side of the hexagonal region and H_1 - L_α transition at right hand side is observed. The surfactant layer curvature is changed to more positive in the H_1 - I_1 transition and to less positive in the H_1 - L_α transition. The water molecules hydrated to the poly(oxyethylene) chain decreases with the increase in the $C_{12}EO_8$ concentration in water (16). Consequently, the effective volume of the hydrophilic chain of $C_{12}EO_8$ decreases and also the repulsion between neighboring hydrophilic chains decreases, which reduces the highly positive curvature. Hence the spontaneous curvature of the surfactant layer in the hexagonal phase is high at relatively low surfactant concentration and low at high concentration. It is reported (1,13,17,18) that the surfactant layer curvature is changed to opposite direction by adding oil depending on the surfactant hydrophilicity or the spontaneous curvature of surfactant layer, in which the highly positive curvature is changed to more positive by the solubilization of oil but the less positive curvature decreases more or it is changed to a negative curvature. Therefore the H_1 - I_1 transition at low surfactant concentration and the H_1 - L_α transition at high surfactant concentration are observed by adding TOG.

3.3 Changes in Microstructure of Liquid Crystals by Adding Oil

SAXS measurement was performed to study the structural change in the liquid crystals by adding oil. An interlayer spacing, d , was obtained from a Bragg peak of a SAXS spectrum. The results are shown in **Fig. 3** for the I_1 , H_1 and L_α phases.

r_1 , r_H , d_L and a_s are calculated from eqs. (1)-(6) and also plotted in **Fig. 3**. For the I_1 phase, we used the constants of a face-centered structure for eqs. (1) and (2). For all single-phase regions of liquid crystals, d , r_1 , r_H and d_L increase as the oil content increases. a_s decreases for the I_1 phase and it is almost constant for the H_1 and L_α phases.

When oil molecules are solubilized in surfactant aggregates, these are partitioned in two sites in the inte-

Fig. 3 Interlayer Spacing, d , for I₁, H₁ and L_α Phases is Plotted against the Weight Fraction of TOG. Effective cross sectional area per surfactant molecule at hydrophile-lipophile interface, a_s , radius of lipophilic core of spherical micelle in I₁ phase, r_I , radius of lipophilic core of cylindrical micelle in H₁ phase, r_H and half thickness of lipophilic bilayer in L_α phase, d_L , are also plotted. The water/C₁₂EO₈ ratio is fixed at (a) 6/4 for I₁ phase, (b) 4/6 for H₁ phase and (c) 28/72 for L_α phase.

rior of surfactant aggregates, i.e. core and palisade layer. In the case of solubilization in core, it is reported that a_s is almost constant for H₁ and L_α phases (1) and decreases for I₁ phase (12), which appear as same trend as in **Fig. 3**. Increasing r_I , r_H and d_L also indicates the oil solubilization in core (1). Hence TOG molecules in the present system are almost solubilized in the core of aggregates.

3.4 Effect of Temperature and Oil Molecular Size on the Cubic Phase

Figure 4 is a phase diagram as functions of weight fraction of TOG and temperature at constant water-to-C₁₂EO₈ ratio (56/44).

An H₁ phase is formed in the oil-free system and the H₁ phase is changed into an I₁ phase by adding a small amount of oil. The melting temperature of the I₁ phase is about 30°C with 3% of oil and it abruptly increases by increasing oil content and reaches up to 43°C. In other words, the cubic phase is stabilized against temperature by solubilizing oil. Similar thermal-stabilization effect for I₁ phase is also observed in other surfactant systems (4,5).

The maximum oil content in the cubic phase and the micellar phase increases by increasing temperature. The spontaneous curvature of surfactant layer decreases by increasing temperature for poly(oxyethylene)-type non-ionic surfactant due to the dehydration from

Fig. 4 Phase Diagram of Water/ $C_{12}EO_8$ /TOG System at Fixed Water/ $C_{12}EO_8$ Ratio, 56/44. Weight fraction of TOG and temperature are variables. An H_1 - I_1 phase transition takes place by adding small amount of TOG to the aqueous $C_{12}EO_8$ system. The melting temperature of the I_1 phase increases with increasing oil content.

poly(oxyethylene) chain (19). Therefore larger micelles can be formed at higher temperature and the larger micelles can solubilize more oil.

Figure 5 shows the position of I_1 phases in the water/ $C_{12}EO_8$ /oil systems.

Three different oils are used, i.e. THG, TOG, TDG. The I_1 phases for the systems with TOG and TDG appear in similar composition range whereas the I_1 phase for the THG system shifts to higher oil content. It is reported that the cubic phase shifts to higher oil content with oil having smaller molecular weight (20) since the amount of oil molecules that penetrate into the surfactant palisade layer in micelles increases, which allows forming larger micelles having smaller surfactant layer curvature. Although these triglyceride oils are mostly solubilized in the core of micelles, oil with smaller molecular weight tends to penetrate more, which causes the difference in the cloud temperature as is shown in **Fig. 1**. The molecular weight of THG molecule is smaller than the other two oils. Therefore the I_1 phase for the THG system is formed with higher oil content. However the I_1 phases of the TOG and TDG system is not so different although the molecular weight is different. The I_1 phase for the TOG system is very close to the aqueous surfactant system (bottom axis).

Fig. 5 Cubic Phase Regions for Water/ $C_{12}EO_8$ /Triglyceride Oil Systems at 25°C are Mapped in a Triangle Composition Diagram. The cubic phase region for the TDG system (shaded area) is included in the cubic phase region for the TOG system.

Therefore, even if the penetration tendency of TOG molecules is higher than the TDG molecules, the I_1 phase cannot shift to lower oil content. In the case of the TDG system, the region of the I_1 phase is shrunk instead of shifting the region.

4 Conclusion

The phase behavior and the microstructures of liquid crystals were studied in water/ $C_{12}EO_8$ /triglyceride oil systems. From the changes in cloud temperatures and effective cross sectional area of surfactant molecule in liquid crystals with varying the oil content, triglyceride oils in this study (1,2,3-trihexanoylglycerol, 1,2,3-trioctanoylglycerol and 1,2,3-tridecanoylglycerol) is mainly solubilized in the core of surfactant self-assemblies, which is similar solubilization behavior as saturated hydrocarbons. Therefore the micellar phase and the hexagonal phase turn into the discontinuous cubic phase by adding these triglyceride oils. The needed amount of oil solubilization to form the discontinuous cubic phase is smaller in the case of triglyceride oil with smaller molecular weight. It was also found that the discontinuous cubic phase stabilized against temperature by the solubilization of oil.

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