

Emulsifying Potency of New Amino Acid-Type Surfactant (II): Stable Water-in-Oil (W/O) Emulsions Containing 85 wt% Inner Water Phase

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The ternary phase diagram for *N*-[3-lauryloxy-2-hydroxypropyl]-L-arginine L-glutamate (C12HEA-Glu), a new amino acid-type surfactant, /oleic acid (OA)/water system was established. The liquid crystal and gel complex formations between C12HEA-Glu and OA were applied to a preparation of water-in-oil (W/O) emulsions. Stable W/O emulsions containing liquid paraffin (LP) as the oil and a mixture of C12HEA-Glu and OA as the emulsifier were formed. The preparation of stable W/O emulsions containing 85 wt% water phase was also possible, in which water droplets would be polygonally transformed and closely packed, since the maximum percentage of inner phase is 74% assuming uniformly spherical droplets. Water droplets would be taken into the liquid crystalline phase (or the gel complex) and the immovable water droplets would stabilize the W/O emulsion system. The viscosity of emulsions abruptly increased above the 75 wt% water phase (dispersed phase). The stability of W/O emulsions with a lower weight ratio of OA to C12HEA-Glu and a higher ratio of water phase was greater. This unusual phenomenon may be related to the formation of a liquid crystalline phase between C12HEA-Glu and OA, and the stability of the liquid crystal at a lower ratio of oil (continuous phase). W/O and oil-in-water (O/W) emulsions containing LP were selectively prepared using a mixture of C12HEA-Glu and OA since the desirable hydrophile-lipophile balance (HLB) number for the emulsification was obtainable by mixing the two emulsifiers.

Key words amino acid-type surfactant; water-in-oil emulsion; liquid crystal; ternary phase diagram; oleic acid; viscosity

Emulsion describes a heterogeneous liquid system in which one liquid is intimately dispersed in another (immiscible) in the form of droplets, and is divided broadly into two types: oil-in-water (O/W) emulsion and water-in-oil (W/O) emulsion. Emulsions are used in many fields; cosmetics,¹⁾ foods,^{2–4)} medicines⁵⁾ and so on. It is thus desirable to prepare emulsions with good stability. Surfactants are used as an emulsifier and/or dispersing agent, and the properties of the emulsion depend on the kind of surfactant. Meanwhile, the effect of surfactant on human skin must be taken into account. Amino acid-type surfactants are biodegradable and have little irritating action on the skin.^{6–8)} We reported the surface active properties of *N*-[3-lauryloxy-2-hydroxypropyl]-L-arginine L-glutamate (C12HEA-Glu), a new amino acid-type surfactant.⁹⁾ Furthermore, the emulsifying potency of C12HEA-Glu in an O/W emulsion system was examined previously¹⁰⁾: the emulsifying potency of C12HEA-Glu was greater than that of popular amphoteric and nonionic surfactants; the stability of emulsions containing oleic acid (OA), (C12HEA-Glu/OA/water system), was greatest.

Good stability of O/W emulsions is attained by choosing a proper oil (dispersed phase) and surfactant (emulsifier).¹¹⁾ On the contrary, stabilization of W/O emulsions is rather difficult compared with O/W emulsions.¹²⁾ One emulsifying technique for preparation of W/O emulsions is a liquid-crystal emulsification. In a previous report,¹⁰⁾ a possible complex formation between OA and C12HEA-Glu on the droplet surface was suggested, which contributes to stabilization of O/W emulsion system.

In this study, formation of liquid crystals between C12HEA-Glu and OA was examined by microscopy under

polarized light and infrared (IR)-spectroscopy, and the ternary phase diagram for C12HEA-Glu/OA/water system was established. W/O emulsions consisting of C12HEA-Glu, OA, liquid paraffin (LP) and water were prepared, and the effects of the amount of water (dispersed phase) and weight ratio of OA to C12HEA-Glu on the stability of W/O emulsions were investigated.

Experimental

Materials C12HEA-Glu was used as an amino acid-type surfactant. The synthesis and purification of C12HEA-Glu were described elsewhere.¹³⁾ OA (99% purity) obtained from Nippon Oil and Fats Co., Ltd. and LP obtained from Wako Pure Chemical Ind., Ltd. were used as supplied. Distilled water for injection was purchased from Otsuka Pharmaceutical Co.

Phase Diagram The ternary phase diagram for C12HEA-Glu/OA/water system was obtained as follows: 3–4 g mixtures were placed in test tubes and heated at 90–100 °C for 1 week; after equilibrium had been established, the mixtures were observed at 25 °C under an inverted microscope.

Optical microscopy Liquid crystals in the C12HEA-Glu/OA/water system were observed under an inverted microscope with a transmitted light differential interference contrast attachment (IMT-2, Olympus Optical Co., Ltd.). Liquid crystalline phase was confirmed under polarized light.

pH Measurement The pH of aqueous C12HEA-Glu solution containing OA was determined with a pH electrode (GST-5311C, TOA Electric Co.) and an ionic meter (1M-40S, TOA Electric Co.) at 25 °C.

IR Spectroscopy Fourier transform (FT)-IR spectrum measurements for C12HEA-Glu, OA and the complex were carried out using the KBr method. The apparatus used was a spectrophotometer FT/IR-400 (JASCO Co.).

Preparation of W/O Emulsions An aqueous C12HEA-Glu solution was added to an LP solution containing OA, and the mixture was stirred for 1 min with a vortex mixer (VORTEX-2-GEMIE, SM Instrument Co.) and then sonicated for 8 min by an ultrasonic instrument (Bransonic 220, Smith-Kline Co.). The concentration of C12HEA-Glu was kept at 1.5 wt%, and the weight ratio of OA to C12HEA-Glu was 3–6. In this study, a lower concentration of emulsifier was used to accelerate the coagulation and coalescence of emulsions so that their measurements would be kinetically convenient.

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Determination of Emulsion Type The type of emulsions prepared was determined by an electric conductivity measurement.

Viscosity A stress-control-type rheometer (Carri-Med CLS 2100, TA-Instruments Co.) was used to measure the viscosities of emulsions. The temperature was controlled at 25 ± 0.1 °C by means of a Peltier element.

Results and Discussion

Ternary Phase Diagram The ternary phase diagram for the C12HEA-Glu/OA/water system was obtained, and is shown in Fig. 1. (Indistinguishable phase is represented by a dotted line.) In the binary C12HEA-Glu/water system, C12HEA-Glu formed micelles up to 30 wt% and changed into an isotropic liquid crystalline phase (I) and liquid crystalline phase (LC) with increasing concentrations of C12HEA-Glu. Next, when OA was added to the binary C12HEA-Glu/water system, OA was solubilized in C12HEA-Glu micelles in a lower C12HEA-Glu concentration region, and a gel phase with high viscosity was formed in a higher C12HEA-Glu concentration region. The gel phase was separated into an oil phase and an LC (O+LC) as the concentrations of OA and C12HEA-Glu increased. It was confirmed by optical microscopy that the LC was a lamellar liquid crystal, which is characterized by a cross-shaped pattern.¹⁴ A lamellar liquid crystal was observed not only in the LC, but also in the oil phase. The (O+LC) changed into a monomeric LC by further adding OA. A fan-shaped pattern was observed in the monomeric LC (Figs. 2b and c). An is also found in other ternary systems: *i.e.* C12HEA-HCl/isostearic acid/water sys-

tem.¹⁵

At above 90 wt% C12HEA-Glu concentration, C12HEA-Glu solidified because of its limited solubility.

Complex Formation between C12HEA-Glu and OA

The change in pH of aqueous C12HEA-Glu solution (1.5 wt%) by adding OA is shown in Fig. 3. The pH decreased with increasing molar ratio of OA, and became constant above a molar ratio of 1, suggesting the interaction between C12HEA-Glu and OA with 1 : 1 molar ratio.

Next, the solid-state interaction between C12HEA-Glu and OA was examined. C12HEA-Glu and OA (1:1 molar ratio) were mixed, melted, and then solidified. The IR spectrum of the mixed compound is shown in Fig. 4. The IR spectra of C12HEA-Glu and OA are also given in Fig. 4. The peak at 940 cm^{-1} for O–H out-of-plane bending vibration of the carboxyl groups of OA disappeared in the mixed compound. The peaks at 1405 and 1470 cm^{-1} for symmetrical and asymmetrical stretching vibrations of carboxylic acid ions were observed in the mixed compound. The IR spectra suggest that the cationic part of C12HEA-Glu electrostatically combines with the anionic part of OA to form a complex. Thus, the formation of an equimolar complex consisting of C12HEA-Glu and OA is strongly suggested from the data shown in Figs. 3 and 4.

We reported previously¹⁰ that the stability of O/W emulsions prepared using C12HEA-Glu and OA was greatest. This finding would be due to the complex formation between C12HEA-Glu and OA at the surface of emulsion droplets. A rigid interface of droplets contributes to good stability of O/W emulsions.

Stabilization of W/O emulsions is rather difficult compared with O/W emulsions.¹² A liquid-crystal emulsification is known as one emulsifying technique. Thus, complex formation between OA and C12HEA-Glu was used to prepare W/O emulsions.

Boundary Region between O/W and W/O Emulsions

Emulsions containing LP (oil phase), C12HEA-Glu and OA (emulsifier) were prepared by varying the amounts of LP and OA, and the boundary region for the formations of O/W and W/O emulsions was examined. The emulsion type depended on the weight ratio of OA to C12HEA-Glu. O/W and W/O emulsions were easily produced at lower and higher OA ratios, respectively. The hydrophile–lipophile balance (HLB) of OA is 1,¹⁶ while that of C12HEA-Glu is a high number because of its high hydrophilicity, although its exact value has not yet been determined. HLB number is expressed as fol-

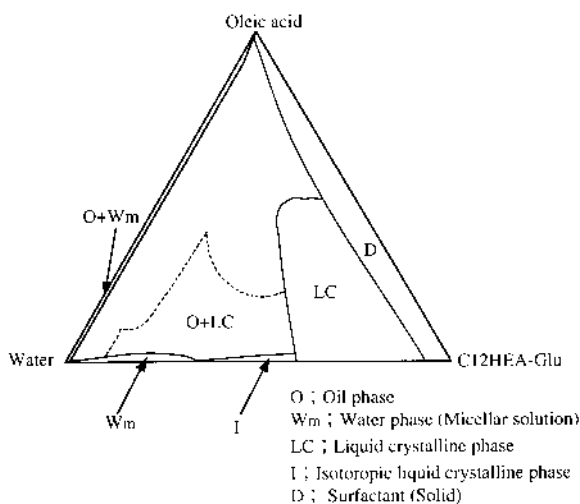


Fig. 1. Ternary Phase Diagram for C12HEA-Glu/OA/Water System

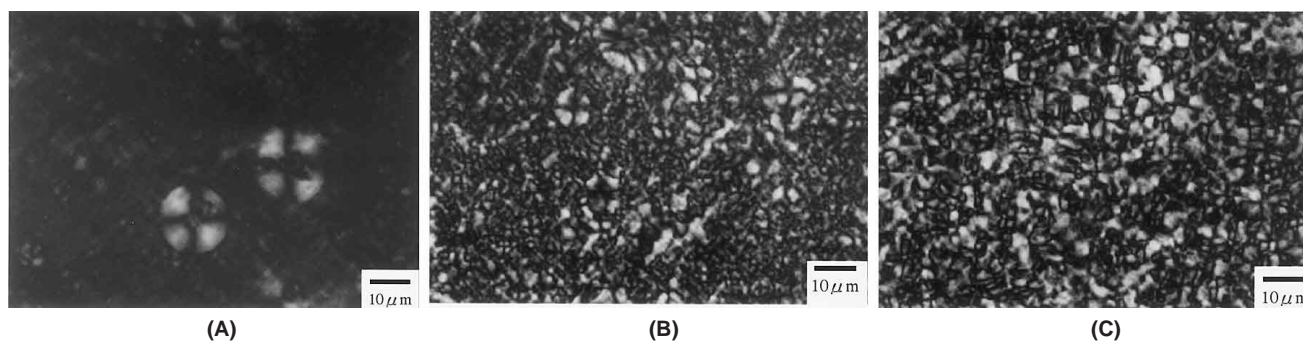


Fig. 2. Optical Micrographs under Polarized Light for LC in C12HEA-Glu/OA/Water System

(a) O+L_α phase (20 wt% C12HEA-Glu); (b) L_α phase (60 wt% C12HEA-Glu); (c) L_α phase (80 wt% C12HEA-Glu).

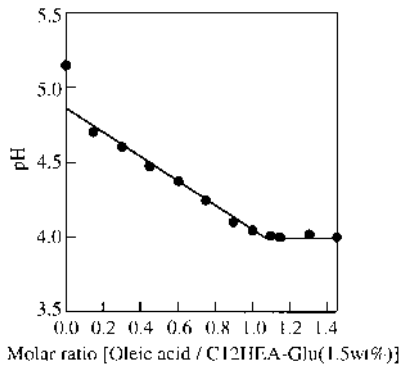


Fig. 3. Changes in pH of Aqueous C12HEA-Glu Solution (1.5 wt%) by Adding OA

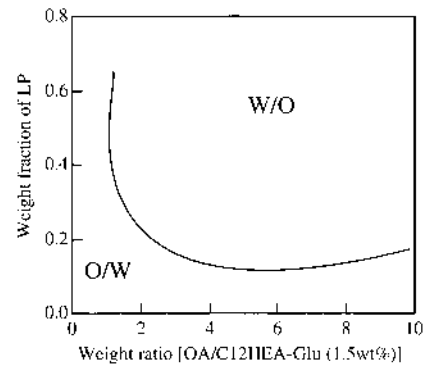


Fig. 5. Boundary Region for the Formations of O/W and W/O Emulsions Containing C12HEA-Glu and OA (Emulsifiers) and LP (Oil Phase)

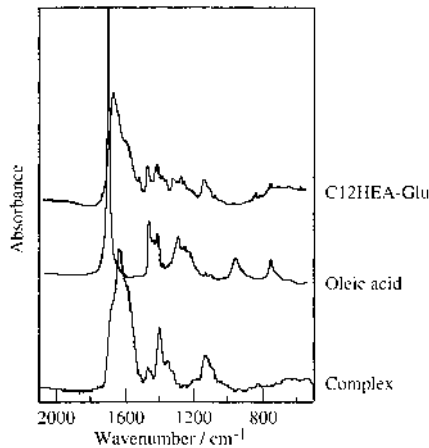


Fig. 4. IR Spectra of C12HEA-Glu, OA and the Equimolar Complex

lows:

$$HLB_{mix} = \sum (HLB_i \times X_i)$$

where HLB_{mix} and HLB_i are the HLB numbers of a surfactant mixture and a single surfactant, respectively, and X_i is the weight fraction of each surfactant. The suitable HLB numbers for emulsification are 4—8 and 8—18 for W/O and O/W emulsions, respectively.¹⁷⁾ Based on the experimental findings, the HLB number of C12HEA-Glu may be estimated at 30—50.

Both W/O and O/W emulsions were produced by using a mixture of C12HEA-Glu and OA as an emulsifier. W/O and O/W emulsions were selectively prepared by changing the weight fraction of OA and C12HEA-Glu. A similar phenomenon has been found for lauroamphoglycinate, an amphoteric surfactant, and OA mixed system.¹⁸⁾

Viscosity of W/O Emulsions W/O emulsions (water/LP system) with various amounts of water were prepared using OA/C12HEA-Glu (3 : 1) as the emulsifier, where the ratio (3 : 1) was chosen taking the boundary region for the formation of W/O emulsions (Fig. 5) and the stability of W/O emulsions (Fig. 7a in the next section) into account. In addition, larger change in viscosity of W/O emulsions by varying the amount of water is shown in the emulsion system containing OA/C12HEA-Glu (3 : 1), this is also the reason for the OA/C12HEA-Glu 3 : 1 ratio. The change in viscosity of W/O emulsions by varying the amount of water (dispersed phase) is shown in Fig. 6. A high viscosity of emulsion im-

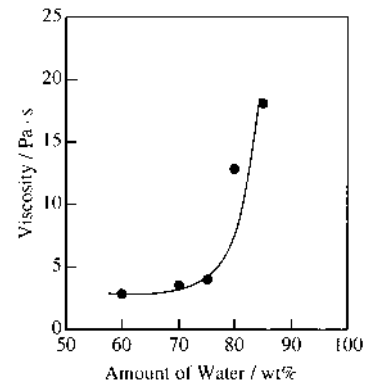


Fig. 6. Effect of Water Phase (Dispersed Phase) on the Viscosity of W/O Emulsions

plies numerous droplets *i.e.* a greater emulsifying state. The viscosity of the emulsions abruptly increased above 75 wt% dispersed water. The maximum volume percentage of the inner phase is 74% assuming uniformly spherical droplets. Emulsion droplets are, however, actually transformable and polydispersable. The droplets of emulsions containing over 74% inner phase are polygonal.^{19,20)} In gel emulsions having over 90 wt% inner phase, each droplet is polygonally transformed and closely packed.²¹⁾ According to the literature,^{19—21)} spherical droplets of W/O emulsions consisting of LP, C12HEA-Glu, OA and water would transform in a polygonal shape above 75 wt% inner water phase.

Stability of W/O Emulsions The effect of weight ratio of OA to C12HEA-Glu on the viscosity of W/O emulsions was examined, where the amount of water was 85 wt%, and the findings are presented in Fig. 7a. The viscosity of emulsions with a lower ratio of OA to C12HEA-Glu was high, the fluidity of the emulsions was extremely low, and the emulsions with the lowest ratio (3) of OA to C12HEA-Glu were most stable. The velocity of creaming in emulsion systems is delayed by increasing the viscosity of emulsions, which is expressed by the Stokes equation, $v = \{d^2g(\rho_p - \rho_m)\}/18\mu$. The delayed creaming would subsequently prevent coagulation and subsequent coalescence.

The phase separation was visually observed at 1 month, 2 d, 1 d and 1 d for emulsions whose weight ratios of OA to C12HEA-Glu were 3, 4, 5 and 6, respectively. The phase separation was accelerated by using a mixed OA/C12HEA-Glu

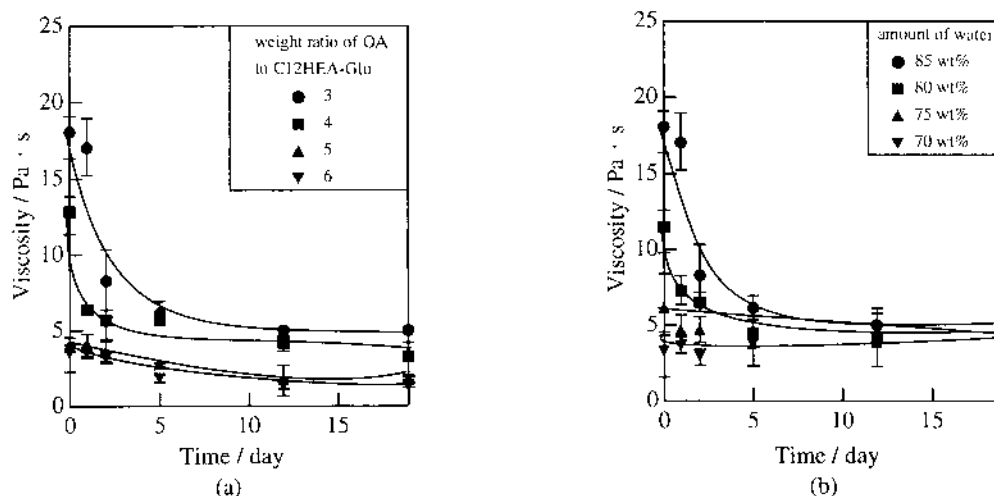


Fig. 7. Time Course of Viscosity of W/O Emulsions

(a) Effect of the weight ratio of OA to C12HEA-Glu on the viscosity of W/O emulsions containing 85wt% water phase; (b) effect of the water phase on the viscosity of W/O emulsions prepared using the mixed emulsifier (OA/C12HEA-Glu=3).

emulsifier having a higher weight ratio of OA to C12HEA-Glu. The viscosity of emulsions decreased with time. This decrease was due to creaming, being coagulation and subsequent coalescence. The number of dispersed droplets decreased by coagulation and coalescence, thereby reducing the viscosity. Coalescence brings about phase separation. Stable W/O emulsions containing LP were prepared by using the emulsifier mixture with a lower weight ratio of OA to C12HEA-Glu. The formation of liquid crystals (complex) composed of C12HEA-Glu and OA would contribute to stabilization of W/O emulsions. The molar ratio of OA to C12HEA-Glu in the complex is 1:1. Excess OA would weaken the interactions between OA and C12HEA-Glu in the liquid crystal (complex), thereby reducing the stability of W/O emulsions containing a higher ratio of OA to C12HEA-Glu. The optimal amount of OA to C12HEA-Glu would be the sum of the required amounts for complex formation between OA and C12HEA-Glu and for W/O emulsification (required HLB number).

There have been reports that the formation of liquid crystals contributes to stabilization of W/O emulsions. Nakama *et al.* reported that water droplets are taken into reversed-hexagonal liquid crystals composed of L-lauryl-*N,N*-dimethyl- α -betaine and OA, and that the immovable water droplets stabilize W/O emulsions.^{18,22,23} Yamashita and Kameyama observed a lamellar liquid crystal surrounding the surface of emulsion droplets.²⁴

Next, the effect of the amount of water (dispersed phase) on the stability of W/O emulsions was examined and the findings are shown in Fig. 7b, where the weight ratio of OA to C12HEA-Glu was 3. The viscosity of emulsion increased with increasing water phase. The phase separation was visually observed at 1 month for emulsions whose inner water phase was 85 wt%, and at 2 d for emulsions whose inner water phase was 70–80 wt%. In general, the stability of W/O emulsions is reduced with increasing ratio of inner water phase.¹⁷ In this study, the stability of W/O emulsions was, however, elevated with increasing ratio of inner water phase. Increasing the oil phase (continuous phase) would bring about a weakening of the LC, since oil penetrates the

gaps between the liquid crystalline layers. Thus, a higher ratio of water phase contributes to stabilization of W/O emulsions containing LP, OA and C12HEA-Glu.

Conclusion

Stable W/O emulsions containing LP as the oil and a mixture of C12HEA-Glu and OA as the emulsifier were formed. The W/O emulsions were stable even though the ratio of water phase (dispersed phase) was over 74 wt%. The stability of W/O emulsions with a lower weight ratio of OA to C12HEA-Glu and a higher ratio of water phase was greater. This would be due to the formation of an LC between C12HEA-Glu and OA, and the stability of the liquid crystal at a lower ratio of oil (continuous phase). Water droplets would be taken into the LC and the immovable water droplets would stabilize the W/O emulsion system.

W/O and O/W emulsions containing LP were selectively obtainable using a mixture of C12HEA-Glu and OA, since the desirable HLB number for the emulsification was achieved by mixing the two emulsifiers.

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