Emulsifying Potency of New Amino Acid-Type Surfactant (1). O/W Emulsions

Junichi KOUCHI*1, Tatsuru TABOASHI*2, Shoko YOKOYAMA*3, Fuminori HARUSAWA*1, Aritomo YAMAGUCHI*1, Hideki SAKAI*1,*4 and Masahiko ABE*1,*4

*1 Faculty of Science and Technology, Science University of Tokyo
(2641, Yamazaki, Noda-shi, Chiba 278-8510, JAPAN)
*2 Ajinomoto Co., Inc., Amino Science Laboratories
(1-1, Suzuki-cho, Kawasaki-ku, Kawasaki-shi, Kanagawa-ken 210-8681, JAPAN)
*3 Kyoritsu College of Pharmacy
(1-5-30, Shibakoen, Minato-ku, Tokyo 105-8512, JAPAN)
*4 Institute of Colloid and Interface Science, Science University of Tokyo
(1-3, Kagurazaka, Shinjuku-ku, Tokyo 162-8601, JAPAN)

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Abstract: Oil-in-water (O/W) emulsions were prepared using a new amino acid-type emulsifier (surfactant), N-[3-lauryloxy-2-hydroxypropyl]-L-arginine L-glutamate (C12HEA-Glu), and the emulsifying potency of C12HEA-Glu was examined in terms of dispersibility, droplet size, interfacial tension, viscosity and hydrophilic microenvironment of droplet surface, whose emulsifying potency was compared with that of general amphoteric and nonionic surfactants. The effects of the constituent oils on the stability of emulsions were also examined.

The emulsifying potency of C12HEA-Glu was greater than that of amphoteric N-lauryl-N, N-dimethyl-α-betaine and nonionic polyoxyethylene(10) lauryl ether, especially, the stability of emulsions containing oleic acid (OA), (C12HEA-Glu/OA/water system), was greatest, while the stability of emulsions containing octane (OC), (C12HEA-Glu/OC/water system), was inferior. OA possesses a hydrophilic carboxyl group and the viscosity of OA is higher than that of OC. The higher visco-elasticity of OA droplet surface and the formation of hydration layer on the droplet contributed to the greater stability of OA emulsions, thereby raising the stability of emulsions for the system C12HEA-Glu/OA/water in spite of the small zeta-potential (insufficiently electrostatic repulsion) of droplets.


Key words: amino acid-type surfactant, oil-in-water emulsion, emulsification, emulsion stability, oleic acid

1 Introduction

Emulsion is a heterogeneous liquid system in which one liquid is intimately dispersed in another liquid (immiscible) in the form of droplets, and is divided broadly into two types: oil-in-water (O/W) emulsion: dispersion of droplets of an organic liquid (an "oil") in an aqueous solution; water-in-oil (W/O) emulsion: dispersion of aqueous droplets in an organic liquid. Emulsions are utilized in many fields, e.g. cosmetics (1), foods (2-4), medicine (5), agricultural chemicals (6) and various manufacturing industries (7). It is then desirable to prepare emulsions with a good stability. Various emulsifying techniques have been developed: liquid-crystal emulsification (8-11), D-phase emulsification (12,13) and phase inversion emulsification (14,15). Physical emulsifying techniques using a high pressure homogenizer (16), microporous glass membrane or glass beads (17) have also been developed. The stability of O/W emulsion is improved by choosing a proper oil (dispersed phase) and surfactant (emulsifier) (18), al-

Corresponding author: Shoko YOKOYAMA
E-mail: yokoyama-sk@kyoritsu-ph.ac.jp
though stabilization of W/O emulsions is rather difficult compared with O/W emulsions (19).

Surfactants are used as an emulsifier and/or a dispersing agent, and the properties of emulsion depend on the kind of surfactant. Recently, fluorocarbonic surfactant (20) and macromolecular surfactant (21,22) have been synthesized and their emulsifying potencies become evident now. Meanwhile, the effect of surfactant on the human skin must be taken into account. Amino acid-type surfactants are biodegradable and have little irritating action on the skin (23-25). We reported the solution properties of N-[[3-lauroxyloxy-2-hydroxypropyl]-L-arginine L-glutamate (C12HEA-Glu), a new amino acid-type surfactant. Thus, in a previous paper (26), we determined the critical micelle concentrations and the micellar aggregation numbers of C12HEA-Glu at various pH values.

In this study, the emulsifying potency of C12HEA-Glu was investigated in terms of dispersibility, droplet size and interfacial tension between oil and aqueous surfactant solution, and the potency revealed was compared with that of general amphoteric and nonionic surfactants.

2 Experimental

2-1 Materials

N-[[3-Lauroxyloxy-2-hydroxypropyl]-L-arginine L-glutamate (C12HEA-Glu) was used as an amino acid-type surfactant. The synthesis and the purification of C12HEA-Glu were reported in a previous paper (27). N-Lauryl-N, N-dimethyl-o-betaine (LB) as an amphoteric surfactant and polyoxyethylene(10) lauryl Ether (POE) as a nonionic surfactant obtained from Japan Surfactant Co. and Japan Emulsion Co., respectively, were used as supplied. Distilled water for injection was purchased from Otsuka Pharmaceutical Co. Oleic acid (OA) (99% purity) obtained from Nippon Oil and Fats Co., Ltd., isopropyl myristate (IPM) and n-octane (OC) obtained from Tokyo Chemical Ind., Ltd., n-hexane (HD) and liquid paraffin (LP) obtained from Wako Pure Chemical Ind., Ltd. were used as supplied.

2-2 Preparation of O/W Emulsions

O/W Emulsions were prepared at 25°C as follows: 3 mM oil was added to an aqueous surfactant solution, and the mixture was stirred for 1 min with a vortex mixer (VORTEX-2-GEMIE, SM Instrument Co.) and then sonicated for 8 min on an ultrasonic instrument (Bransonic 220, Smith-Kline Co.). It is reported (28) that emulsification efficiency with ultrasonication method is almost the same as that with high-speed rotary homogenizer. Concentrations of surfactants were kept 1/5 the critical micelle concentration (cmc) of each surfactant (C12HEA-Glu, 100 μM; LB, 30 μM; POE, 5 μM). In some studies, concentrations of surfactants are kept about the cmc of surfactant (saturated adsorption at the air/water interface) for emulsification. In this study, the concentration of surfactant (1/5 the cmc of surfactant) was chosen to accelerate the coagulation and coalescence rates so that their measurement would be kinetically convenient. In addition, we have recently found the formation of surfactant-free O/W emulsions with a good stability (29). Thus, a higher concentration of surfactant would not be necessarily required.

2-3 pH Measurement

The pH of emulsion was determined with a pH electrode (GST-5311C, TOA Electric Co.) and an ionic meter (1M-40S, TOA Electric Co.) at 25°C.

2-4 Observation of Emulsions

Emulsions were observed under an inverted microscope with a transmitted light differential interference contrast attachment (IMT-2, Olympus Optical Co., Ltd.).

2-5 Freeze-Fracture Electron Microscopy

Emulsion sample was frozen immediately after preparation in liquid nitrogen. Frozen sample was fractured in a freeze-replica apparatus (FR-7000A, Hitachi Science Co.) at −150°C. Replica was made by platinum-carbon shadowing followed by platinum shadowing. It was cleaned with acetone and distilled water, and then picked up on 150 mesh copper grids and observed with an electron microscope (JEM-1200EX, Japan Electron Co.).

2-6 Measurement of Droplet Size

Droplet size of emulsions was determined by a dynamic light scattering method. The distribution and time course of droplet size were measured at 25°C using a NICOMP 380 ZLS (Particle Sizing System Co.). The light source was a diode pump solid state laser (DPSS laser) with a wavelength of 535 nm and the scattering angle was 90°.

2-7 Measurement of Turbidity

Change in the turbidity of emulsion with time was
measured at 700 nm using a spectrophotometer (MPS-2000, Shimadzu Co.).

2.8 Interfacial Tension at Oil/Water Interface

Interfacial tension at oil/water interface was measured with a Wilhelmy-type autotensiometer (CBVP-Z, Kyowa Interface Science Co.) equipped with a platinum plate. After an equilibrium had been attained, the value of interface tension was obtained. Specific gravities of oils and aqueous surfactant solutions, which are parameters needed to evaluate interfacial tensions, were measured with a hydrometer (DA-210, Kyoto Electronics Co.).

2.9 Zeta-Potential of Droplets

Zeta-potential of droplets in emulsions was measured using a zetasizer (NICOMP 380 ZLS, Particle Sizing System Co.), which is a laser Doppler electrophoresis apparatus. 20 mW laser and 5.0 V/cm were applied during electrophoresis and the scattering angle was 19.8°.

2.10 Viscosity

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

2.11 Fluorescence Spectrum

A solution of 1×10⁻⁴ mol L⁻¹ of ANS as a fluorescent probe was added to sample emulsion. Fluorescence spectrum was measured with a spectrofluorometer (RF-5000, Shimadzu Co.) equipped with a pump-circulating water at a constant temperature through an outer tube. The temperature was maintained at 25±0.2°C. The wavelength of excitation was 337 nm.

3 Results and Discussion

3.1 Dissociation State of C12HEA-Glu in Emulsions

C12HEA-Glu possesses amino and carboxyl groups in the molecule. The dissociation state of C12HEA-Glu depends on the pH of emulsion. Thus, the pH of the emulsion composed of C12HEA-Glu solution and oil was measured at first. The oils used were OA, OC, IPM and HD. The concentrations of C12HEA-Glu and oil were 0.1 mM and 3 mM, respectively. The results are indicated in Table 1. The pH value of emulsions ranged between 4 and 6. Taking into account the pKa (26) of C12HEA-Glu, the arginine part of C12HEA-Glu would be positively charged and the glutamate part is negatively charged as shown in Fig. 1.

<table>
<thead>
<tr>
<th>pH</th>
<th>OA</th>
<th>OC</th>
<th>IPM</th>
<th>HD</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.4</td>
<td>5.3</td>
<td>5.8</td>
<td>5.7</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 1 Structural Formula of C12HEA-Glu in pH 4~6 Emulsion Solutions.

3.2 Formation of Emulsions

O/W Emulsions consisting of oil (OA, OC, IPM or HD) and surfactant solution (C12HEA-Glu, LB or POE) were prepared. The droplet diameter of emulsions just after preparation was 100~200 nm in all cases. Figure 2 is a typical electron micrograph of freeze-fracture replicas of OA emulsion (C12HEA-Glu/OA/water system). Emulsions with 100~150 nm droplet diameters were observed, and the droplet size determined from electron micrographs was well consistent with that determined by the dynamic light scattering method.

3.3 Stability of Emulsions

The effect of surfactants (C12HEA-Glu, LB, POE) on the stability (dispersibility) of emulsions prepared using OA, OC, IPM or HD as an oily dispersed phase was examined by measuring the time course of change in droplet size. Emulsions without any surfactant were also supplied as a reference. The time course of change in droplet diameter for OA emulsions is shown in Fig. 3 (a).

The droplet diameter of the emulsions just after preparation was 100~200 nm in all cases. The droplet size of the emulsions prepared with no surfactant increased to 1 μm (1000 nm) in 3 d. The times required for droplets to grow up to micro-order size ones were 1 w, 1.5 months and 2 months for the emulsions prepared with POE, LB
and C12HEA-Glu, respectively, indicating that the stability of OA emulsions becomes greater with C12HEA-Glu.

The time course of droplet size change for IPM emulsions is shown in Fig. 3(b). The droplet diameter of the emulsions just after preparation was 200–300 nm. Droplets of the emulsions having no surfactant grew up to micro-order size ones in 2 w. The times required for droplets to grow up to 1 μm size ones were 3 w, 1 month and 1.5 months for the systems emulsified with POE, LB and C12HEA-Glu, respectively. The stability of IPM emulsions prepared using C12HEA-Glu was greatest as in the case of OA emulsion systems.

The time course of droplet size change for OC emulsions is shown in Fig. 3(c). The droplet diameter of the emulsions just after preparation was approximately 500 nm and the droplet size immediately increased to 1.2 μm in a few hours in all cases. No significant difference was observed in stability between emulsions prepared using various kinds of surfactants.

The time course of droplet size change for HD emul-

![Fig. 2 Electron Micrograph of Freeze-Fracture Replica Prepared from OA Emulsions Prepared Using C12HEA-Glu.](image)

**Fig. 3** Time Course of Droplet Size of Emulsions Prepared Using C12HEA-Glu, LB and POE.

Oil phase: (a), OA; (b), IPM; (c), OC; (d), HD.
sions is shown in Fig. 3(d). The droplet diameter of HD emulsions just after preparation was 200～300 nm. The droplet size of the emulsions having no surfactant increased to 1 μm in 2 months. Droplets with 200～300 nm sizes in HD emulsions prepared with POE, LB and C12HEA-Glu became 1 μm size ones in 3, 3 and 4 months, respectively. The stability of HD emulsions was greater than that of OC emulsions, due probably to the longer alkyl chain length of HD than OC. The stability of emulsions with no surfactant containing saturated hydrocarbon compound has been found to increase with increasing hydrocarbon chain length (30). A similar result was obtained in our emulsion systems containing surfactant.

The relative stability of emulsion prepared using surfactant was defined as \( t_c/t_w \), where \( t_c \) and \( t_w \) are the times required for droplets to grow up to 1 μm size ones in emulsions with and without surfactant, respectively. The relative stabilities of emulsions are summarized in Table 2.

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Relative Stability of Emulsions.</th>
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<tbody>
<tr>
<td></td>
<td>Water</td>
</tr>
<tr>
<td>OA</td>
<td>1.0</td>
</tr>
<tr>
<td>IPM</td>
<td>1.0</td>
</tr>
<tr>
<td>OC</td>
<td>1.0</td>
</tr>
<tr>
<td>HD</td>
<td>1.0</td>
</tr>
</tbody>
</table>

The relative stability of emulsions containing HD and C12HEA-Glu (C12HEA-Glu/HD water system) was 2.0, while that of emulsions consisting of OA and aqueous C12HEA-Glu solution (C12HEA-Glu/OA/water system) was 20. The stability of HD emulsions was greater than that of OA emulsions as shown in Fig. 3 (a) and (d), while the relative stability of OA systems emulsified with C12HEA-Glu was greater than that of HD systems emulsified with the same surfactant as indicated in Table 2. The relative stabilities of OC and IPM emulsions were 1.0 and 1.5～3.2, respectively. On the contrary, the relative stability of OA emulsions prepared with C12HEA-Glu was 20, implying that the stability of OA emulsions is improved 20 times by emulsifying with C12HEA-Glu. The emulsifying potency of C12HEA-Glu was excellent in OA emulsions. Recent papers have reported that the dispersibility of unstable emulsions containing no surfactants is improved by adding a small amount of oils such as hexadecane and squalene (29,31,32). In this study, we attempted to stabilize OC emulsions, which are extremely unstable as shown in Fig. 3(e), by adding OA (OC : OA = 10 : 1). These emulsions are abbreviated hereafter to “OC/OA emulsions”. The time course of change in droplet size for OC/OA emulsions is shown in Fig. 4.

The diameter droplets in OC/OA emulsions just after preparation was 200～300 nm. The droplet size of the emulsions having no surfactant increased to 1 μm in 1 d. The times required for droplets to grow up to microorder size ones were 3 d, 10 d and 2 w for the emulsions prepared using POE, LB and C12HEA-Glu, respectively. The relative stabilities of the emulsions prepared with POE, LB and C12HEA-Glu were 3, 10 and 14, respectively, as shown in Table 3.

<table>
<thead>
<tr>
<th>Table 3</th>
<th>Relative Stability of Emulsions.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Water</td>
</tr>
<tr>
<td>OC/OA = 10/1</td>
<td>1.0</td>
</tr>
</tbody>
</table>

The stability of OC/OA emulsions improved by the presence of C12HEA-Glu as in the case of OA emulsions.

3.4 Turbidity of Emulsions
The time course of turbidity change for OA emulsions is shown in Fig. 5.

The initial turbidity of emulsions containing surfactant was higher than that of emulsions without surfactant, implying a larger number of droplets consisting of surfactant and OA. Furthermore, the turbidity of OA emulsion containing C12HEA-Glu was highest, suggesting a higher emulsifying potency of C12HEA-Glu compared with the other surfactants. The turbidity slightly
increased first and then decreased with time. The decrease in turbidity would be due to creaming, which is caused by a rising of oily droplet to the surface. Emulsion without surfactant visually brought about a coalescence after a few days, although the turbidity measurement was not carried out beyond 3 h.

3.5 Effect of Interfacial Tension on Stability of Emulsions

The interfacial tensions between oils and aqueous surfactant solutions are indicated in Table 4.

The effect of oils on the interfacial tension was examined at first. The interfacial tensions were in the order, OA < IPM < OC/OA < OC < HD, with respect to the oils used. The lowest interfacial tension for OA would be due to the hydrophilic property of OA possessing a carboxyl group. On the contrary, the interfacial tension for HD was highest because of its high hydrophobicity. The initial droplet size in emulsions is said to be smaller at lower interfacial tension (32, 33). However, no significant difference was found in droplet size between OA and HD emulsions. Next, the effect of surfactants on the interfacial tension (oil / aqueous surfactant solution interface) was examined. The interfacial tension was in the increasing order, C12HEA-Glu < LB < POE, with respect to the surfactants. No significant difference was also found in droplet size emulsions prepared using the surfactants. Thus, the stability of emulsion can not be discussed in terms of interfacial tension.

<table>
<thead>
<tr>
<th>Oil</th>
<th>Interfacial tensions (mN⋅m⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Water</td>
</tr>
<tr>
<td>OA</td>
<td>10.6</td>
</tr>
<tr>
<td>IPM</td>
<td>16.3</td>
</tr>
<tr>
<td>OC/OA=10/1</td>
<td>22.7</td>
</tr>
<tr>
<td>OC</td>
<td>48.8</td>
</tr>
<tr>
<td>HD</td>
<td>51.6</td>
</tr>
</tbody>
</table>

3.6 Effect of Viscosity on Stability of Emulsions

The viscosities of the oils (Table 5) were in the order, OC < OC/OA < IPM < HD < OA. The stability of emulsion increased in the order, OC < OC/OA < IPM < HD < OA, as shown in Figs. 3 and 4, which is consistent with the order of the oily viscosity. The interfacial visco-elasticity of OA droplets is greater than that of the other oily droplets because of the high viscosity of OA. OA emulsions are not likely to bring about a coalescence even if a coagulation occurred, whereas OC emulsions are likely to bring about a coalescence through a coagulation because of the lower visco-elasticity of OC droplets. The viscosity of oil affected the emulsion stability.

Table 5 Viscosity of Oils.

<table>
<thead>
<tr>
<th>Oil</th>
<th>Viscosity/MPa⋅s</th>
</tr>
</thead>
<tbody>
<tr>
<td>OA</td>
<td>29.4</td>
</tr>
<tr>
<td>HD</td>
<td>2.86</td>
</tr>
<tr>
<td>IPM</td>
<td>2.45</td>
</tr>
<tr>
<td>OC/OA=10/1</td>
<td>0.782</td>
</tr>
<tr>
<td>OC</td>
<td>0.607</td>
</tr>
</tbody>
</table>

3.7 Zeta-Potential of Droplets in Emulsions

The zeta-potential of droplets in emulsions and its time course of change are shown in Fig. 6.

The zeta-potential of droplets in emulsions having no surfactant was negative and its absolute value decreased with time. The absolute value of the zeta-potential of droplets in OA emulsions was largest, owing to the dissociated carboxyl groups of OA. Droplets in the other emulsions prepared using oils possessing no carboxyl group also exhibited negative zeta-potentials, due probably to hydrophobic hydration (adsorption of OH⁻ on droplet surfaces) (34,35).

Jayme et al. (36) have reported that a 37.5 mV zeta-
potential (absolute value) was necessary to prevent aggregation of droplets and stabilize emulsion system. The observed zeta-potentials \((-25 \sim -35 \text{ mV})\) of droplets in emulsions having no surfactant are then insufficient to stabilize them. The zeta-potential of droplets in emulsions prepared using C12HEA-Glu was positive (Fig. 6 b), owing to the cationic form of C12HEA-Glu (Fig. 1) in an acidic medium. The positive zeta-potential of droplets in the emulsions increased with time. This would be due to the increasing adsorption amount of C12HEA-Glu on the droplet surface with time. The absolute values of the zeta-potential of OC and OA droplets in emulsions were largest and smallest, respectively. Since OA molecule possesses a carboxyl group, the electric charges on OA droplets and C12 HEA-Glu molecules would neutralize each other to give the zeta-potential to the droplets. The stability of OA emulsion was great, although the zeta-potential of OA droplets was 15 mV, smaller than 37.5 mV (absolute value). This would be due to the high viscosity of OA: namely, the interfacial visco-elasticity of OA droplets is high, thereby OA emulsions would not bring about a coalescence through a coagulation. In addition, a rigid interface of the droplet caused by the adsorption of C12 HEA-Glu on OA droplet surface would contribute to a good stability of OA emulsions. A possible interaction between OA and C12HEA-Glu will be investigated in a subsequent study.

The zeta-potentials of droplets in emulsions prepared using LB and POE (Fig. 6 c and d) were negative as in surfactant free emulsions (Fig. 6 a). Because LB and POE are an amphoteric and a nonionic surfactants, respectively, the effect of LB and POE on the zeta-potential of droplets in emulsions was insignificant. LB and POE may contribute to stabilize emulsion systems through their adsorption on the droplet surface in emulsions.

### 3.8 Interfacial State of Droplets in Emulsions by ANS Probe

The hydrophobic microenvironment of droplets in emulsions was investigated by means of the ANS fluorescent method. ANS scarcely fluoresces in an aqueous bulk phase, while it strongly fluoresces when transferred to a hydrophobic environment \(37,38\). The fluorescence intensity of ANS was high in emulsion compared with that in aqueous solution. ANS adsorption on the droplet surface in emulsions was confirmed. The fluorescence spectra of ANS are shown in Fig. 7.

The fluorescence intensity of ANS in emulsion decreased and the maximum wavelength of ANS redshifted with time, implying that the hydrophobicity of
droplet surfaces just after emulsion preparation is highest and that the surface state of droplets becomes hydrophilic with time. The change in fluorescence intensity of ANS with time in OA emulsion system was largest. The surface of OA droplets probably becomes hydrophilic with time. The fluorescence intensity of ANS in HD emulsion system was highest, suggesting the most hydrophobic surface of HD droplets. One of factor that prevent aggregation of droplets in emulsion is the electrostatic repulsion between charged droplets, and the other one is a hydration on the droplet surface. In OA emulsions hydration layers are probably formed on the droplet surface since the hydrophilic property of OA emulsions is relatively high. The hydration layer on the droplet surface would contribute to stabilize emulsion system.

4 Conclusion
The emulsifying potency of C12HEA-Glu was greater than that of LB and POE. The stability of OA emulsions prepared using C12HEA-Glu was greatest. This would be due to the high visco-elasticity of OA droplets and a formation of hydration layer on the droplet surface to make the stability of OA emulsion great although the absolute value of the zeta-potential of OA droplets in emulsions prepared using C12HEA-Glu was small.
References