Study on Preparation and Formation Mechanism of *n*-Alkanol/Water Emulsion Using α-Cyclodextrin

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Surfactants are usually used for the preparation of emulsions; however, some have an adverse effect on the human body such as skin irritation, hemolysis, and protein denaturation, *etc.* In this study, we examined the preparation and formation mechanism of *n*-alkanol/water emulsions using α-cyclodextrin (α-CD) as an emulsifier. Emulsions were prepared by mixing oil and water phases for 4 min at 2500 rpm using a vortex mixer. The mechanism of emulsification was investigated with some physico-chemical techniques. From phase diagrams of *n*-alkanol/α-CD/water systems, the emulsion phase extended as the chain length of *n*-alkanols and the amount of α-CD added increased. Furthermore, the emulsion was not formed in the region where the *n*-alkanol/α-CD complex didn't precipitate; however, the emulsion was formed in the region where the complex precipitated. In addition, it was clear that the emulsions have a yield stress value and correspond to the Maxwell model from rheological measurement. Our experiments clearly showed that the stable emulsions are formed because the precipitated complexes form a dense film at the oil–water interface and prevent aggregation among dispersed phases. Furthermore, it is suggested that the creation of a three-dimensional network structure formed by precipitated complexes in the continuous phase contributes to the stabilization of the emulsion. Thus, we concluded that the *n*-alkanol/water emulsions using α-cyclodextrin were a kind of the Pickering emulsion.

Key words Pickering; emulsion; cyclodextrin; *n*-alkanol; complex; rheology

Emulsion is widely applied in many pharmaceuticals such as injections, ointments, and suppositories, *etc.* However, emulsion is a thermodynamically unstable liquid/liquid dispersed system that causes phase separation *via* four processes of creaming, aggregation, coalescence, and Ostwald ripening. So, the best way to stabilize the emulsion is through the addition of an emulsifier. An emulsifier is an amphiphilic substance with intramolecular hydrophilic and hydrophobic groups, and surfactant is generally used as an emulsifier. Surfactant is usually used to enhance emulsification and make the emulsion formed stable. However, some surfactants have adverse effects of skin irritation, hemolysis, and environmental pollution, *etc.*[1,2] On account of these problems, the preparation of emulsion without the need for surfactant or through the addition of substances instead of surfactant would be ideal.

A few studies on emulsion preparation that used cyclodextrins (CDs) as an emulsifier have been done.[3—8] CD is an α-1,4 linked cyclo-oligosaccharide composed of glucopyranose units. The three major CD homologs, α-CD, β-CD, and γ-CD, consist of six, seven, and eight glucopyranose units, respectively. The unique property of CDs is that they have a hydrophilic shell and a relatively apolar cavity. It is well known that CDs form inclusion complexes with various insoluble substances through host-guest interactions. Moreover, CDs show excellent biodegradability, and their high-level safety has been confirmed in chronic and acute toxic tests.[9—13] Studies on emulsion using CDs have been carried out from the viewpoint of the preparation of emulsion; however, the formation mechanism of the emulsion has hardly been reported.

In this study, we examined the preparation and formation mechanisms of *n*-alkanol/water emulsions using α-CD in detail.

Experimental

Materials α-CD was purchased from Nihon Shokuhin Kako Co., Ltd. (Tokyo, Japan) and used after drying in a vacuum. *n*-Alkanols (*n*-hexanol, *n*-heptanol, *n*-octanol, and *n*-decanol) were purchased from Kanto Chemical Co., Inc. (Tokyo, Japan) and used without further purification. Distilled water per injection JP (Japanese Pharmacopoeia) was obtained from Otsuka Pharmaceutical Co., Ltd. (Tokyo, Japan).

Preparation of *n*-Alkanol/Water Emulsions Using α-CD Emulsions were prepared as follows: α-CD solution (concentration: 2.5—10.0 w/v %) and *n*-alkanol were placed in test tubes with screw caps and sealed. The test tubes were warmed at 25 °C for 10 min, and mixed thoroughly for 4 min at 2500 rpm using a vortex mixer (REAX top, Heidolph Instruments GmbH & Co., Schwabach, Germany).

Phase Diagram Phase diagrams of *n*-alkanol/α-CD/water systems were obtained using visual and microscopic observations at 1 h after preparation. The emulsion type (O/W or W/O) was determined using dye and dilution tests.

Interfacial Tension Each *n*-alkanol (60 ml) was accumulated on the α-CD solution (20 ml) of various concentrations and left at 25 °C for 1 h. The interfacial tension of *n*-alkanol/α-CD solutions was measured with the Wilhelmy type surface tensiometer (CBVP-A3, Kyowa Interface Science Co., Ltd., Saitama, Japan) at 25 °C.

Powder X-Ray Diffraction Powder X-ray diffraction measurement of the precipitates obtained by centrifuging the emulsion was carried out. Homogenous emulsions were centrifuged at 5000 rpm for 2 h using a centrifugal separator (KOKUSAN-SUPERIOR, Kokusan Co., Ltd., Tokyo, Japan), and the obtained precipitates were dried at 50 °C for 3 h. The powder X-ray diffraction patterns were taken by a Rigaku RINT 1400 diffractometer with Ni-filtered Cu-$K\alpha$ radiation.

Amount of Precipitated *n*-Alkanol/α-CD Complex The amount of precipitated *n*-alkanol/α-CD complex in emulsion was calculated from the mass balance measurement of the α-CD concentration in the supernatant solution after the emulsion was centrifuged. This supernatant solution was then analyzed by high-performance liquid chromatography (HPLC) under the following conditions: pump, EYELA PLC-5D (Tokyo Rikakikai Co., Ltd., Tokyo, Japan); detector, Shodex RI-71 refractive index detector (Showa denko K.K., Tokyo, Japan); column, TSK gel Amide-80 (250×4 mm i.d., Tosoh Co., Tokyo, Japan); mobile phase, acetonitrile/water (6 : 4, v/v); flow rate, 1.0 ml/min.

Polarized Light Microscopy The interface of *n*-alkanol/water emulsions was observed using a polarized light microscope (ECLIPSE 600W

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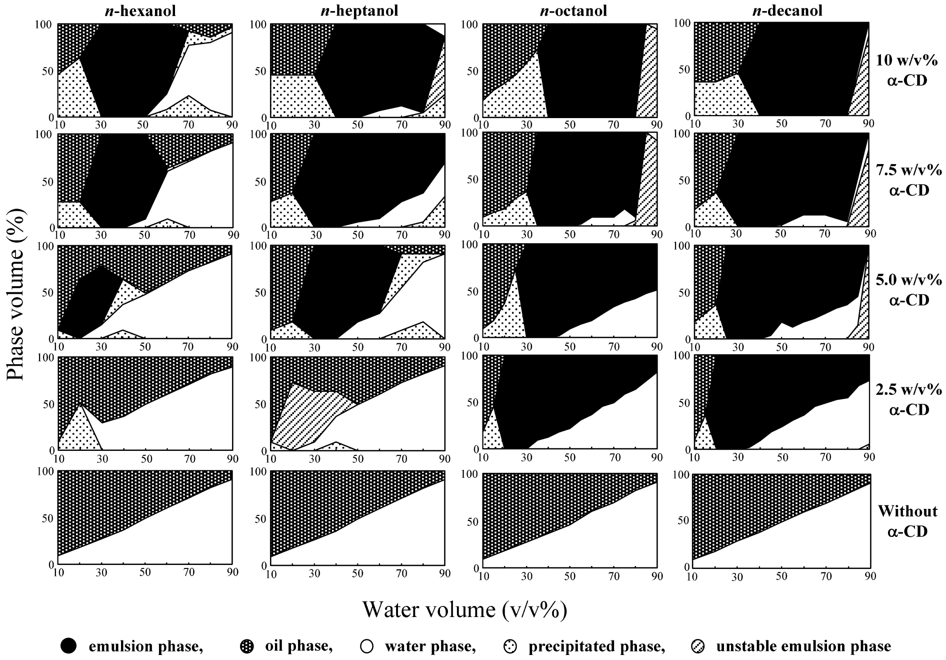

Fig. 1. Variations in Phase Behavior of *n*-Alkanol/α-CD/Water Systems at 25 °C

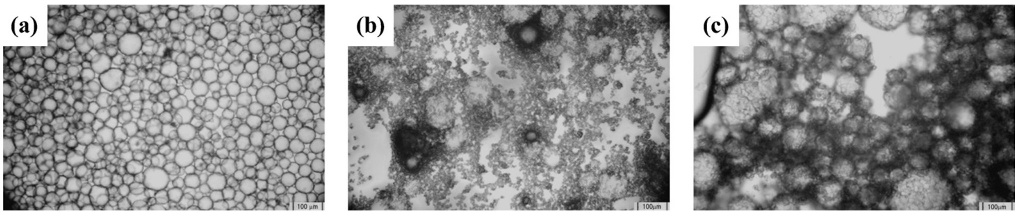

Fig. 2. Microphotographs of *n*-Alkanol/α-CD/Water Systems

(a) Emulsion phase, (b) precipitated phase, and (c) unstable emulsion phase.

POL, Nikon Co., Tokyo, Japan).

Droplet Size Measurement The droplet size of *n*-alkanol/α-CD emulsions was calculated with particle size distribution analysis software (Mac-View ver. 3.5, MOUNTECH Co., Ltd., Tokyo, Japan). This software calculates the droplet size from emulsion photographs and converts into the particle size distribution.

Stability Test Stability of *n*-alkanol/water emulsions was evaluated by measuring the time course of the volume fraction of the emulsion phase at 25 °C. The volume fraction of the emulsion phase was obtained by the height ratio between the emulsion phase and total phases.

Rheological Measurement Dynamic viscoelasticity measurements of *n*-alkanol/water emulsions were performed using a stress-controlled rheometer RheoStress 600 (HAAKE GmbH, Karlsruhe, Germany) equipped with a parallel-plate geometry (diameter, 35 mm) at 25 °C. The frequency ranged from 0.06 to 188 rad/s. The linear viscoelasticity region was determined through stress sweep tests at a fixed frequency (6.28 rad/s).

Results and Discussion

Phase Diagrams of *n*-Alkanol/α-CD/Water Systems Figures 1 and 2 show the phase behavior of ternary components of *n*-alkanol/α-CD/water systems and the microphotographs of each phase, respectively. No *n*-alkanol/water systems could produce emulsion in the absence of α-CD. However, the emulsion phase appeared when α-CD was added, and the emulsion particles were homogeneous (see Fig. 2a). The emulsion phase extended as the chain length of *n*-alkanols and the amount of α-CD added increased. In the region where the ratio of oil in the systems was large, the precipitated phase was mainly formed (see Fig. 2b). On the other hand, in the region where the ratio of water in the systems was large, an unstable emulsion phase that consisted of emulsion and precipitates was formed (see Fig. 2c). Therefore, it was suggested that the precipitates took part in the formation of the emulsion.

Figure 3 shows the powder X-ray diffraction patterns of precipitates in *n*-alkanol/α-CD/water systems. It was strongly suggested that the precipitates were complexes of *n*-alkanol and α-CD, considering that all X-ray diffraction patterns of precipitates obtained from *n*-alkanol/α-CD/water systems were totally different from that of the free α-CD.

Next, we examined the amount of precipitated complexes in *n*-alkanol/water emulsions. Figure 4 shows the relationship between the amount of precipitated complexes and the total α-CD concentration in *n*-alkanol/α-CD/water systems. The amount of precipitated complexes was markedly elevated with the increasing hydrocarbon chain length of *n*-alkanol. The formation of precipitates was initiated by the addition of smaller amount of α-CD to the alkanols of a longer hydrocarbon chain. The solubility of the complexes into water thus decreased in the order of the chain length of alkanols, *i.e.* *n*-hexanol$>$*n*-heptanol$>$*n*-octanol$>$*n*-decanol. In addition, from Fig. 1, the emulsion was not formed in the region where the *n*-alkanol/α-CD complex did not precipitate; however, the emulsion was formed in the region where the complex precipitated.

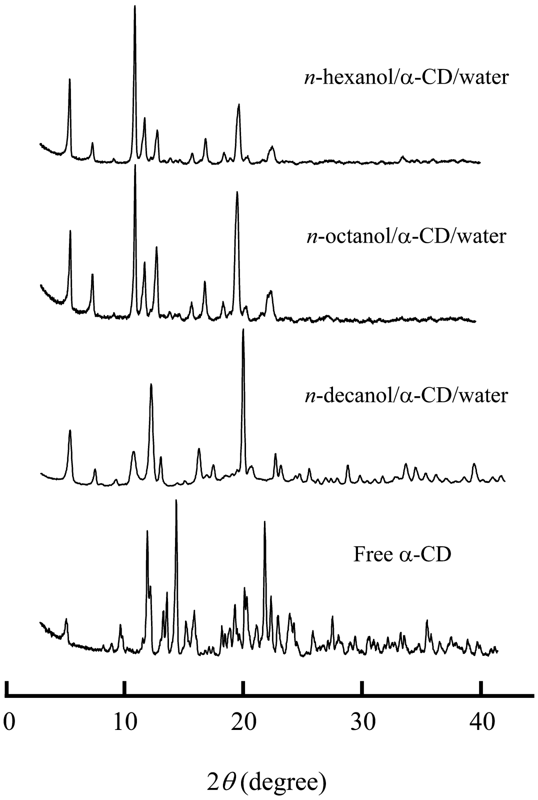

Fig. 3. Powder X-Ray Diffraction Patterns of Precipitates in *n*-Alkanol/α-CD/Water Systems

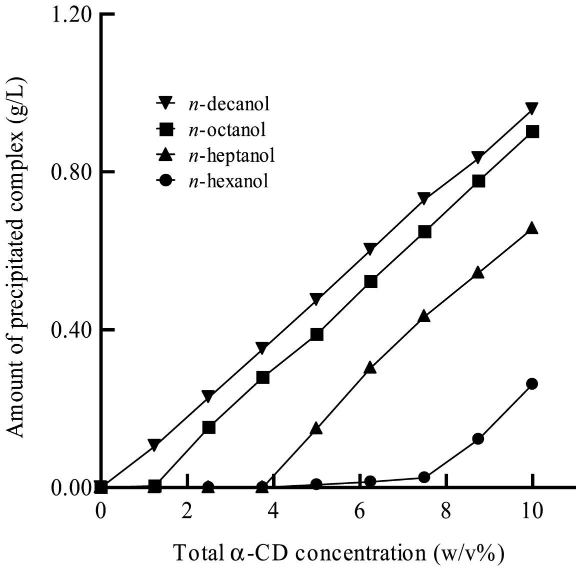

Fig. 4. Relationship between the Amount of Precipitated Complexes and the Total α-CD Concentration

Water in *n*-alkanol/water systems is 80 v/v%.

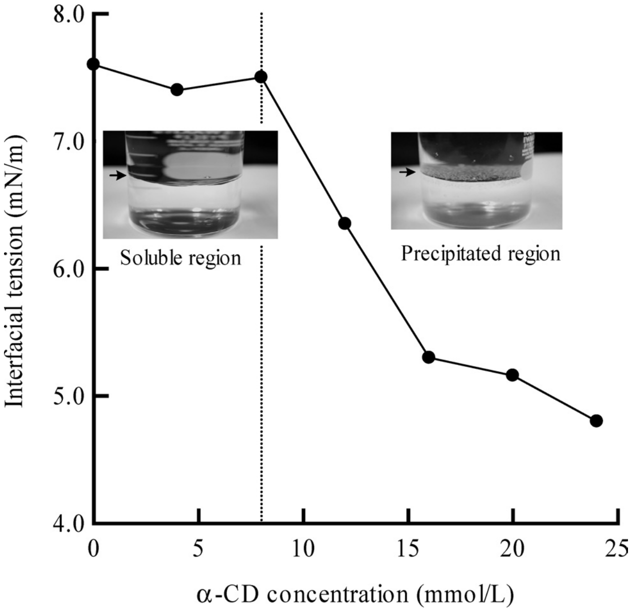

Fig. 5. Effects of the α-CD Concentration on the Interfacial Tension of the *n*-Octanol/Water Interface at 25 °C

The inset shows photographs of the *n*-octanol/water interface. The arrow in the insets shows the position of the oil–water interface.

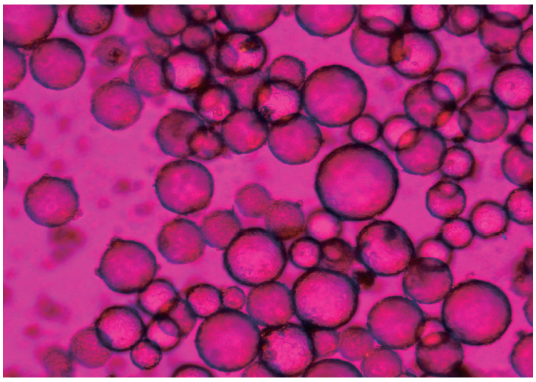

Fig. 6. Microphotograph of *n*-Octanol/Water Emulsion Using α-CD Taken under Crossed Nicol Polarization

Surface-Activity of Precipitated *n*-Alkanol/α-CD Complex To ascertain the surface-activity of the *n*-alkanol/α-CD complex, the interfacial tension of *n*-alkanol/α-CD solution was measured. Figure 5 shows the relationship between the interfacial tension of *n*-octanol/α-CD solution and the α-CD concentration as an example. In the region of low α-CD concentration, the interfacial tension of *n*-octanol/α-CD solution was not significantly changed by the addition of α-CD. On the other hand, in the region of high α-CD concentration, the interfacial tension was markedly reduced with increasing α-CD concentration. In addition, we examined the interfacial condition of both concentration regions, and it was found that the precipitated layer of the complex was formed at the oil–water interface in the region of high α-CD concentration (see inset of Fig. 5). From these results, it was considered that the interfacial tension of the oil–water interface was decreased with increasing α-CD concentration because the measurement was hampered by the formation of the precipitated layer at the oil–water interface. Therefore, it was clarified that the precipitated complex had surface activity, though the soluble complex had little surface activity.

The surface state of *n*-alkanol/water emulsion droplets was investigated using polarized light microscopy. Figure 6 shows the microphotograph of *n*-octanol/water emulsion as an example. From the photograph, it was confirmed that the precipitated complex was adsorbed to the droplet surface because polarized light that originated in the precipitated complex was observed on the surface of oil droplets. That is to say, these precipitated complexes were adsorbed to the

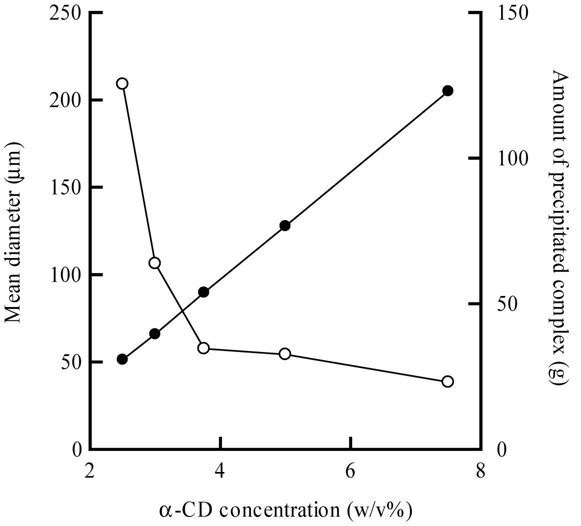

Fig. 7. Effects of the α-CD Concentration on the Droplet Size of Emulsion and Amount of Precipitated *n*-Octanol/α-CD Complex

oil–water interface and they were acted as a dispersing agent for the stabilization of emulsion. Pickering reported that a steady emulsion could be prepared using insoluble solid particles such as $CuSO_4$ and $Fe(OH)_2$ as emulsifiers.[14)] Subsequently, a variety of solid materials have been used as emulsifiers to prepare emulsions in many studies.[15—24)] These emulsions that use solid materials as emulsifiers are called Pickering emulsions. Thus, we thought that the *n*-alkanol/water emulsions using α-CD were a kind of Pickering emulsion.

Stability of Emulsions The droplet size of an emulsion is one of the most important parameters to evaluate its stability. The relationship between the droplet size of an emulsion (d) and the creaming rate (v) is defined by the Stokes equation:

$$v=\frac{d^2(\rho_s-\rho_0)g}{18\eta_0} \tag{1}$$

where ρ_s and ρ_0 are the density of the particle and medium, respectively, g is the acceleration of gravity, and η_0 is the viscosity of the medium. From Eq. 1, if the droplet size of the emulsion is small, it is thought to be a stable emulsion. Figure 7 shows the droplet size of emulsion and amount of precipitated *n*-octanol/α-CD complexes as a function of the α-CD concentration. The droplet size of the *n*-octanol/α-CD emulsion was rapidly decreased with increasing α-CD concentration, and it was unchanged afterwards. However, the amount of precipitated *n*-octanol/α-CD complex was increased with increasing α-CD concentration. The reason why the droplet size of the *n*-octanol/water emulsion decreases by the addition of α-CD is that the surface of oil droplets is covered with the precipitated complexes. Furthermore, it was suggested that the oil droplet size of emulsion was unchanged at the region of high α-CD concentration because the amount of adsorption of the precipitated complex to the surface of oil droplets didn't increase by the addition of CD anymore.

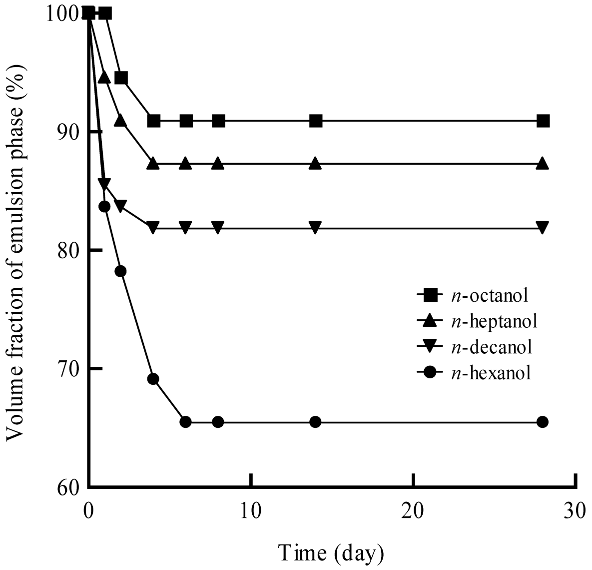

Fig. 8. Evaluation of the Stability of *n*-Alkanol/Water Emulsion Using α-CD

Water: 45 v/v%, α-CD concentration: 10 w/v%.

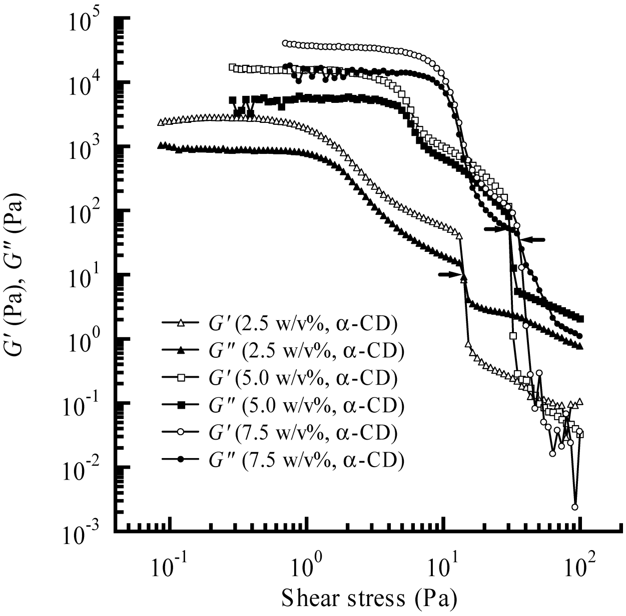

Fig. 9. Shear Stress Dependence of G' and G'' for *n*-Octanol/Water Emulsion Using α-CD

The stability of *n*-alkanol/water emulsions was investigated. Figure 8 shows the time-course of the volume fraction of the emulsion phase. The stability of *n*-alkanol/water emulsions was in the order of *n*-octanol>*n*-heptanol>*n*-decanol≫*n*-hexanol. This order corresponds to the amount of precipitated complexes in *n*-alkanol/water emulsion shown in Fig. 4, except for *n*-decanol/water emulsion. From this, it was suggested that the stability of emulsion is closely correlated with the amount of precipitated complexes at the oil–water interface.

Rheological Behavior of Emulsions Figure 9 shows the shear stress dependence of the storage modulus (G') and loss modulus (G'') for *n*-octanol/water emulsion as an example. This emulsion had a linear viscoelasticity region in which G' and G'' didn't change through alterations in shear stress; that is to say, it is a region where the internal structure of the emulsion doesn't degrade even if the shear stress is changed.

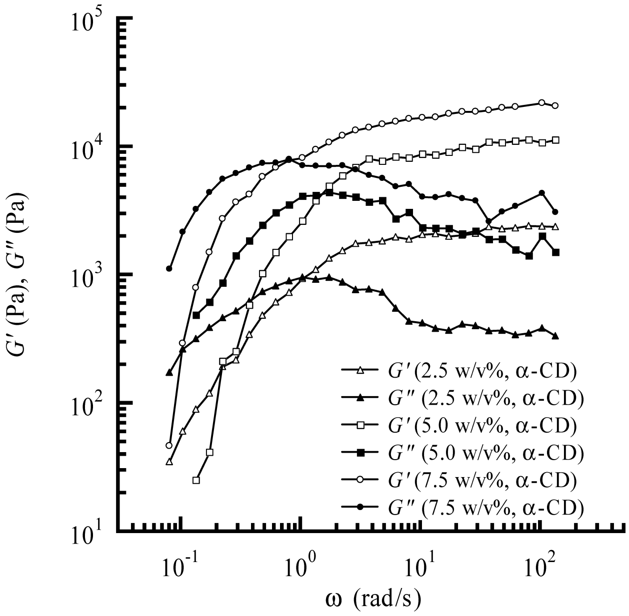

Fig. 10. Frequency Dependence of G' and G'' for *n*-Octanol/Water Emulsion Using α-CD

These systems showed a solid-like behavior ($G'>G''$) in the low shear stress region, and liquid-like behavior ($G'<G''$) was observed in the high shear stress region. The intersection of G' and G'' indicates the yield stress value (see arrows in Fig. 9). The yield stress values of *n*-octanol/water emulsions were increased depending on the α-CD concentration. Furthermore, G' and G'' of *n*-octanol/water emulsions in the linear viscoelasticity region were elevated with increasing α-CD concentration. These results indicate that a three-dimensional network structure formed by the precipitated *n*-alkanol/water complex in the emulsion becomes stronger as the α-CD concentration increases. In addition, the variation of G' and G'' from the end of the linear viscoelasticity region to the yield stress value indicates the breakdown process of the three-dimensional network structure.

To ascertain the higher-order structure of this emulsion in further detail, dynamic viscoelasticity measurements of *n*-alkanol/water emulsions were carried out. Figure 10 shows the variation of G' and G'' as a function of the oscillatory shear frequency (ω) as an example. These systems showed a liquid-like behavior ($G'<G''$) in the low frequency region and solid-like behavior ($G'>G''$) in the high frequency region. From these results, this viscoelastic behavior of *n*-alkanol/water emulsions corresponded to the Maxwell model, which is a basic model of a viscoelastic body. Furthermore, G' and G'' of *n*-octanol/water emulsions were increased with rising α-CD concentration. The cause of this result is similar to the case of the stress-dependence measurement. Furthermore, Midmore reported the rheograms of emulsions made with silica particles, and that emulsions had yield stress values that originated from the interaction between the silica particles.[19] This result strongly supports our opinion that the yield stress value of *n*-alkanol/water emulsions originates from the interaction between the precipitated *n*-alkanol/α-CD complexes.

Formation Mechanism of *n*-Alkanol/Water Emulsion Using α-CD From the above results, we propose two necessary conditions for the stabilization of emulsions by the precipitated *n*-alkanol/α-CD complexes. As the first, the precipitated *n*-alkanol/α-CD complexes should adsorb the oil–water interface and form a dense film on the surface of the dispersed phase. As the second, the precipitated *n*-alkanol/α-CD complexes need to form a three-dimensional network structure in the continuous phase.

Finkle *et al.*[15] and Binks *et al.*[21,23] reported the relationship between the contact angle (θ_{ow}) of solid particles and the emulsion type (O/W or W/O). That is, the solid particles of low θ_{ow} ($\theta_{ow}<90°$) will be held at the oil–water interface and tend to stabilize the O/W emulsion. On the other hand, the solid particles of high θ_{ow} ($90°<\theta_{ow}$) stabilize the W/O emulsion. In this study, the contact angle of the precipitated *n*-alkanol/α-CD complexes was not measured, but it was guessed that the contact angles of the precipitated complexes were less than 90° because the O/W type emulsion had been formed in all cases. In addition, it was suggested that the α-CD part of the *n*-alkanol/α-CD complex was mainly localized on the surface part of the precipitated complexes like a hydrophilic group of surfactant micelles. We concluded that the *n*-alkanol/water emulsions using α-CD were a kind of Pickering emulsion.

Based on the findings obtained from this research, the value of CD-emulsions with practical oils will be dealt with in a following paper.

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