Formation and Characterization of Emulsions Using *β*-Cyclodextrin as an Emulsifier

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Received December 6, 2007; accepted February 12, 2008; published online February 18, 2008

The preparation and characterization of *n*-alkane/water emulsions using *β*-cyclodextrin (*β*-CD) were studied. The prepared *n*-alkane/water emulsions were of the oil-in-water (O/W) type, and the stability of emulsions was in the order of *n*-hexadecane>*n*-dodecane>*n*-octane. From observations using polarized light microscopy and powder X-ray diffraction measurement, it was suggested that the formation of a dense film at the oil–water interface and the three-dimensional structural network created by precipitated complexes in the continuous phase are associated with the stability of emulsion. Furthermore, it was clarified that O/W-type emulsions were formed because the contact angle (θ_{ow}) which the precipitate makes with the interface was $\theta_{ow}<90°$ in all compounds (oils) used in this study.

Key words *β*-cyclodextrin; *n*-alkane; emulsion; precipitate; contact angle; stability

Cyclodextrin (CD) is cyclic oligomer of dextrose joined by *α*-1,4 linkages. CD has a hydrophobic cavity and forms an inclusion complex with hydrophobic guest molecules. CD has been widely used to improve the solubility, stability, and taste, *etc.*, of drugs in pharmaceutical application.[1] However, there are a few reports on the use of CD as an emulsifier. Only the emulsification of fatty acid using CDs by Shimada *et al.*[2,3] and the preparation of O/W/O-type multiple emulsion using CDs by Yu *et al.*[4,5] have been reported. Moreover, the mechanism of emulsion formation using CD has not been sufficiently investigated.

We have recently reported the preparation and formation of *n*-alkanol/water emulsions using *α*-CD as an emulsifier.[6] In this study, we proposed two necessary conditions for the formation of stable emulsion: adsorption to the oil–water interface of precipitated *n*-alkanol/*α*-CD complexes, and the formation of a three-dimensional structural network by precipitates in the continuous phase. The oil phase (*n*-alkanols) used in the previous paper[6] has a hydrophilic group and low interfacial tension. On the other hand, most oil phases for practical use in pharmaceuticals and cosmetics have marked hydrophobicity and interfacial tension, such as liquid paraffin and squalane. Consequently, emulsification using apolar oil should be investigated.

In this paper, we examined the formation and characterization of *n*-alkane/water emulsion using *β*-CD, which is less soluble in water and expected to yield more insoluble precipitates than *α*- and *γ*-CD.

Experimental

Materials *β*-CD was purchased from Nihon Shokuhin Kako Co., Ltd. (Tokyo, Japan) and used after drying in a vacuum. *n*-Alkanes (*n*-octane, *n*-dodecane, and *n*-hexadecane) were purchased from Kanto Chemical Co., Inc. (Tokyo, Japan) and used without further purification. Other reagents were of the purest grade supplied by Wako Pure Chemical Industries Ltd. (Tokyo, Japan). Distilled water for injection JP (Japanese Pharmacopoeia) was obtained from Otsuka Pharmaceutical Co., Ltd. (Tokyo, Japan).

Preparation of Emulsion and Phase Diagram The composition with different levels of *β*-CD, *n*-alkane, and water was placed in a sealed container and mixed at 10000 rpm for 5 min using a homogenizer (Excel Auto Homogenizer, Nihon Seiki Seisakusyo Co., Tokyo, Japan) at 25 °C. Phase diagrams of *n*-alkane/*β*-CD/water systems were obtained using visual and microscopic observations 1 h after preparation. The type of emulsion was determined by electric conductivity measurement and dilution testing.

Powder X-Ray Diffraction Powder X-ray diffraction measurement of the precipitate obtained by centrifuging the emulsion was carried out. The emulsion was centrifuged at 10000 rpm for 30 min using a centrifugal separator (Himac CR20B3, Hitachi Co., Ltd., Tokyo, Japan). After centrifugation, the obtained precipitate was dried at 50 °C in a vacuum. Powder X-ray diffraction measurement of the precipitate was the same as that described previously.[6]

Contact Angle The emulsions were prepared using various compounds as the oil phase. The weight ratio of oil/*β*-CD/water was 0.40/0.08/0.52. The precipitate obtained by centrifuging the emulsion was molded into a pellet-like shape and immersed in oil. Two microliters of water was dropped onto the pellet, and the contact angle (θ_{ow}) which the precipitate makes with the oil–water interface at 25 °C was imaged using a micro camera.[7] The contact angle was then determined by an image analysis system (Visual Scalar MCP-550, Moritex Co., Tokyo, Japan). The contact angle measurements were repeated three times.

Droplet Size Measurement and Stability Test The droplet size and distribution of *n*-alkane/water emulsions were determined by particle size distribution analysis software according to the previous procedure.[6] The stability of *n*-alkane/water emulsions was evaluated by measuring the time-course of the volume fraction of the emulsion phase at 25 °C in a similar manner to that in the previous paper.[6]

Rheological Measurement Dynamic viscoelasticity measurement of *n*-alkane/water emulsions was performed using a stress-controlled rheometer (RheoStress 600, HAAKE GmbH, Karlsruhe, Germany) equipped with parallel-plate geometry (diameter, 35 mm; gap distance, 1.0 mm) at 25 °C. The shear stress dependence of the storage modulus (G') and loss modulus (G'') for emulsions was examined at a fixed frequency (1.0 rad/s). The frequency dependence of G' and G'' for emulsions was measured at a fixed shear strain (2×10^{-4}) in the linear viscoelasticity region.

Results and Discussion

Phase Diagram of *n*-Alkane/*β*-CD/Water Systems O/W emulsions were formed by the addition of *β*-CD to the *n*-alkane/water system. From this, it was clear that *β*-CD can act as an emulsifier. Figure 1 shows phase diagrams of the ternary components of *n*-alkane/*β*-CD/water systems. In the phase diagrams, 'O/W (S)' is a stable O/W emulsion, 'O/W (U)' is an unstable O/W emulsion, 'O' is the separated oil phase, and 'P' is the precipitate. The region of the stable O/W emulsion phase extended as the chain length of *n*-alkanes increased.

Figure 2 shows the microphotographs (a—c) and the microphotograph under crossed nicol polarization (d) for each phase in the *n*-hexadecane/*β*-CD/water system as an example. In the emulsion phase, the *n*-hexadecane droplets in the

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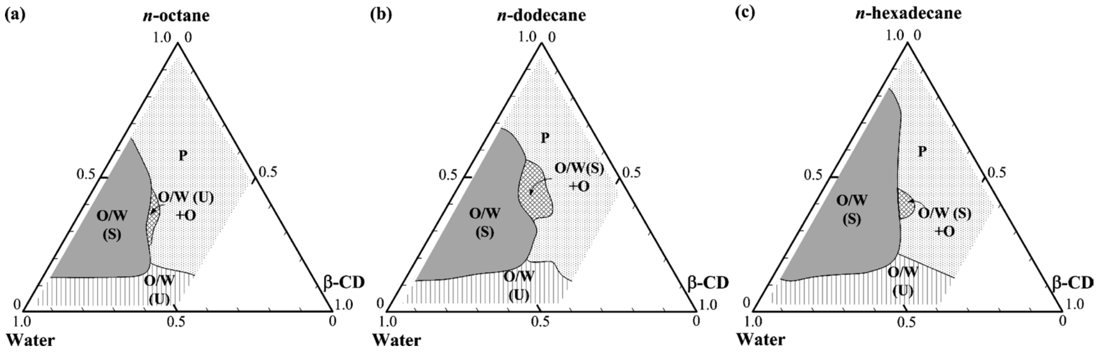

Fig. 1. Phase Diagrams of *n*-Alkane/*β*-CD/Water Systems at 25 °C

(a) *n*-Octane/*β*-CD/water system, (b) *n*-dodecane/*β*-CD/water system, and (c) *n*-hexadecane/*β*-CD/water system.

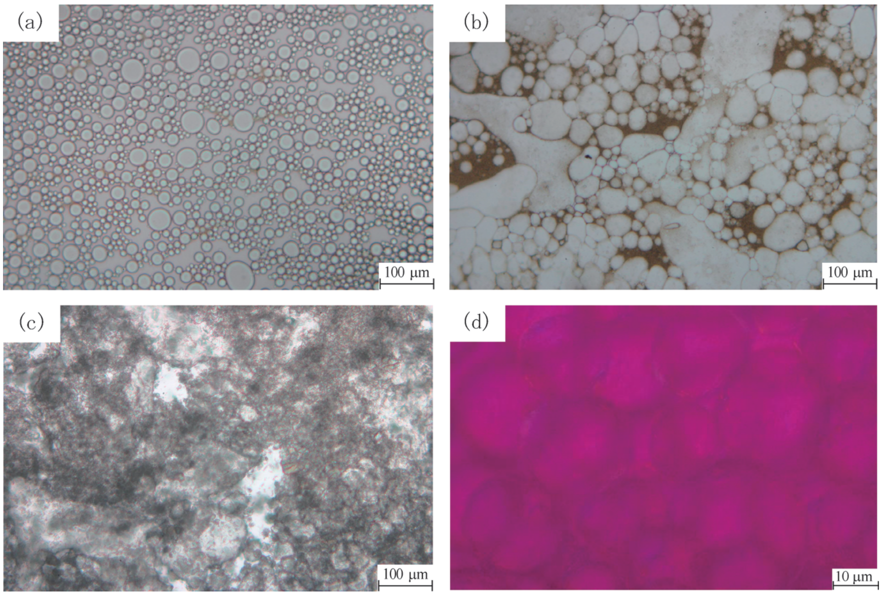

Fig. 2. Microphotographs of *n*-Hexadecane/*β*-CD/Water System

(a) Stable emulsion phase, (b) unstable emulsion phase, (c) precipitated phase, and (d) stable emulsion phase taken under cross nicol polarization.

emulsion were homogeneous (see Fig. 2a). In the unstable emulsion phase, the size of *n*-hexadecane droplets in emulsion was larger than in the stable emulsion, and many elliptically shaped droplets were observed (see Fig. 2b). In the region where the water volume in the system was relatively low, many precipitates were confirmed (see Fig. 2c). Furthermore, it was suggested that the precipitates contribute to the formation of the emulsion because polarized light derived from precipitates on the surface of oil droplets was observed (see Fig. 2d).

Powder X-Ray Diffraction The oil, water, and precipitate were separated when the emulsion was mechanically disrupted by centrifugation. We mixed the separated oil and water in the same manner as in the preparation method for emulsion, but the emulsion was not formed in the absence of precipitates. Thus, the presence of precipitates would seem essential for the formation of emulsion. Next, the separated precipitates were dried out, and powder X-ray diffraction measurements were carried out. The powder X-ray diffraction patterns of precipitates are shown in Fig. 3. It was found that each X-ray diffraction pattern of precipitates in *n*-alkane/*β*-CD/water systems was different from that of free *β*-CD. Also, the diffraction patterns of precipitates agreed very closely with those of the pure *n*-alkane/*β*-CD complexes. Consequently, these precipitates must be inclusion complexes of *β*-CD and *n*-alkane. From the results of microphotographs

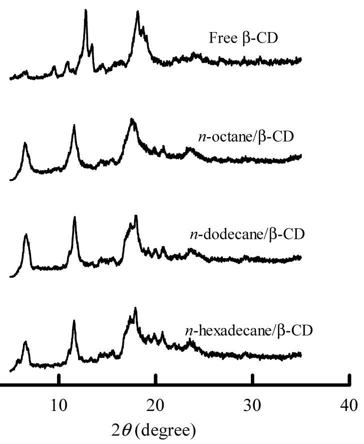

Fig. 3. Powder X-Ray Diffraction Patterns of Precipitates in *n*-Alkane/*β*-CD/Water Systems

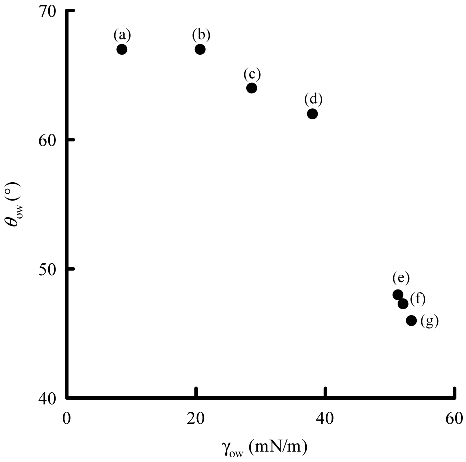

Fig. 4. Relationship between the Contact Angle (θ_{ow}) Which Precipitate Makes with the Interface and Interfacial Tension (γ_{ow}) of Various Compounds Used as an Oil Phase at 25 °C

(a) *n*-Octanol, (b) methyl *n*-octanoate, (c) isopropyl myristate, (d) poly(dimethylsiloxane), (e) *n*-octane, (f) *n*-dodecane, and (g) *n*-hexadecane.

under crossed nicol polarization and powder X-ray diffraction measurement, it was strongly suggested that the precipitated complexes of β-CD and *n*-alkane adsorb at the oil-water interface and stabilize the O/W emulsion.

Contact Angle Surfactants are usually used for the preparation of emulsion. Small, solid particles also stabilize emulsion, and emulsions prepared by solid particles are called Pickering emulsions.[8] The *n*-alkane/β-CD/water emulsions are a kind of Pickering emulsion, because the precipitated complex act in the formation of emulsion as an emulsifier. The type of emulsion is governed by the contact angle (θ_{ow}) which the solid particle makes with the interface.[9—11] That is to say, a solid particle of $\theta_{ow}<90°$ stabilizes O/W emulsion, and that of $\theta_{ow}>90°$ stabilizes W/O emulsion. Also, it is known that θ_{ow} varies with the oil–water interfacial tension (γ_{ow}) of the compound used as the oil phase.[12] We investigated the correlation between θ_{ow} which precipitate makes with the interface and γ_{ow} of various compounds. As a result, θ_{ow} values decreased with increasing γ_{ow} and were less than 90° (Fig. 4). Consequently, it became apparent that the prepared emulsion using β-CD only forms the O/W-type.

Droplet Size and Stability of Emulsions Effects of the β-CD concentration on the droplet size of *n*-alkane/water emulsions were examined. The size distribution of *n*-hexadecane/water emulsion as an example is shown in Fig. 5a. The emulsions generated were polydispersed forms. The droplet size of emulsions was decreased with increasing β-CD concentration (see Fig. 5b). Furthermore, the droplet size of emulsions at the same β-CD concentration was in the order of *n*-octane>*n*-dodecane>*n*-hexadecane. Similarly to *n*-alkanols, the droplet size of emulsions may be related to the amount of precipitated complexes adsorbed to the surface of droplets.[6]

Next, the stability of *n*-alkane/water emulsions was investigated. Figure 6 shows the time-course of the volume fraction of the emulsion phase. The stability of *n*-alkane/water emulsions was in the order of *n*-hexadecane$\gg$*n*-dodecane>*n*-octane. It is thought that the *n*-hexadecane/β-CD/water system forms the most stable emulsion on considering Stokes equation,[13] because the density of *n*-hexadecane is the highest and the droplet size of the formed emulsion is the smallest compared with other *n*-alkanes.

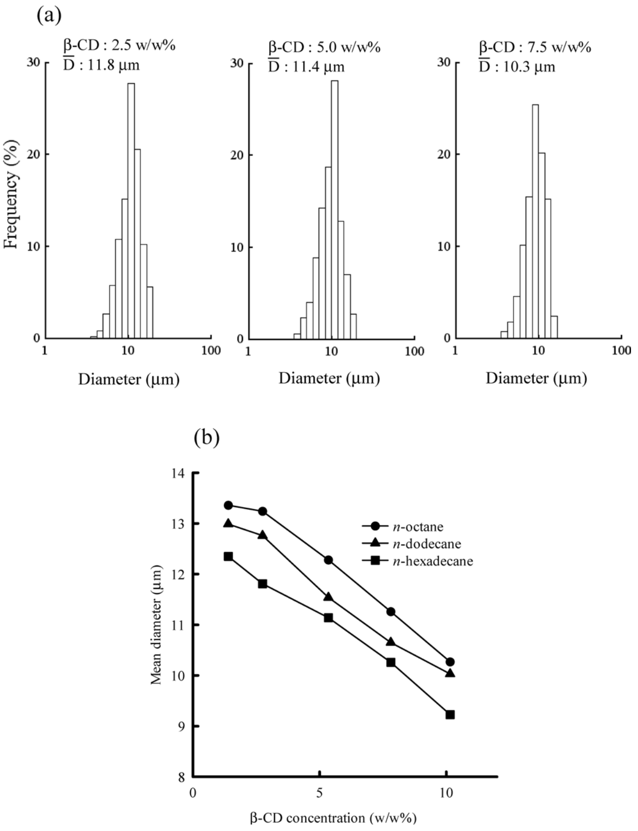

Fig. 5. Effects of β-CD Concentration on the Droplet Size of *n*-Alkane/Water Emulsions

(a) Size distribution, and (b) mean diameter.

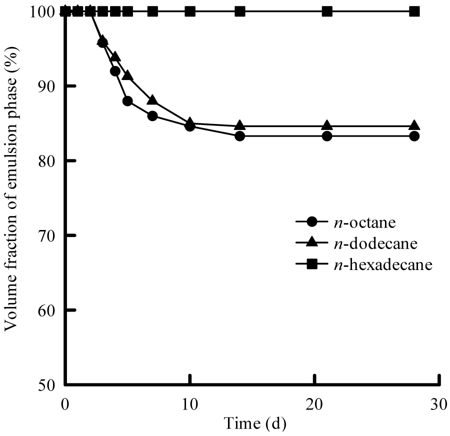

Fig. 6. Evaluation of the Stability of *n*-Alkane/Water Emulsions

Weight ratio of *n*-alkane/β-CD/water is 0.40/0.08/0.52.

Viscoelasticity of Emulsions In order to investigate the mechanism of emulsion stability, the viscoelasticity of emulsion was analyzed. Figure 7 shows the shear stress dependence of the storage modulus (G') and loss modulus (G'') for *n*-alkane/water emulsions. These emulsions exhibited a linear viscoelasticity region in which G' and G'' do not change through alterations in shear stress. G' was elevated with increasing chain length of *n*-alkane. The intersection of G' and

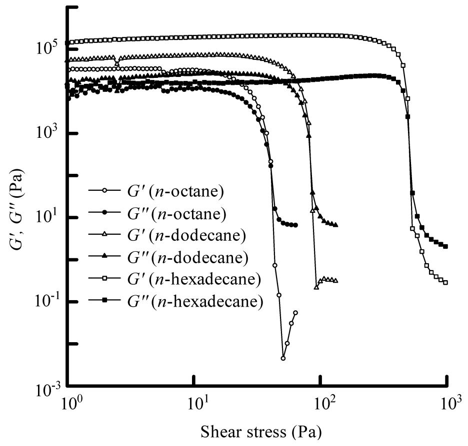

Fig. 7. Shear Stress Dependence of G' and G'' for Various *n*-Alkane/Water Emulsions

Weight ratio of *n*-alkane/β-CD/water is 0.40/0.08/0.52.

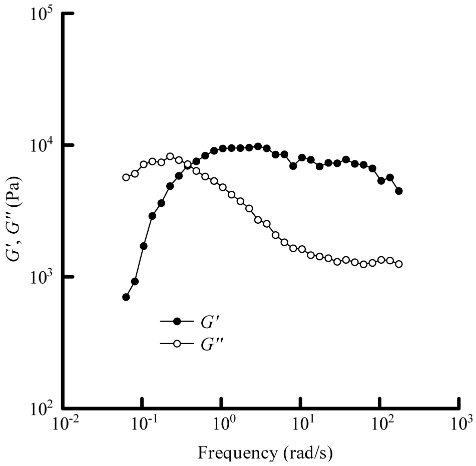

Fig. 8. Frequency Dependence of G' and G'' for *n*-Hexadecane/Water Emulsion

Weight ratio of *n*-hexadecane/β-CD/water is 0.40/0.08/0.52.

G'' indicates the yield stress value of emulsion, and these values were increased depending on the chain length of *n*-alkane. These results indicate that the interaction between the precipitated *n*-alkane/β-CD complexes becomes stronger as the chain length of *n*-alkane increases.

Figure 8 shows the variation of G' and G'' as a function of the oscillatory shear frequency of *n*-hexadecane/water emulsion as an example. This system showed liquid-like behavior ($G' < G''$) at the low and solid-like behavior ($G' > G''$) at the high frequency region. From these results, it was found that this emulsion corresponds to the Maxwell model.

Conclusion

We have investigated the formation of an emulsion consisting of *n*-alkane, water, and β-CD as an emulsifier. As a result, it was suggested that the formation of a dense film at the oil–water interface and a three-dimensional structural network by precipitated complexes derived from CD in the continuous phase are necessary for the formation of a stable emulsion. Furthermore, it was found that the type of emulsion is governed by the contact angle which the precipitated complex makes with the interface.

The present results of this paper may be a fundamental guide to emulsification using CDs, however, further experiments are necessary to assess the usefulness of CD as an emulsifier. Additional studies on emulsification using oil applied in pharmaceuticals and cosmetics will be dealt with in a following paper.

Acknowledgments This work was supported in part by "High-Tech Research Center" Project for Private Universities: matching fund subsidy from MEXT (Ministry of Education, Culture, Sports, Science and Technology), 2007 and Nihon University Research Grant for 2007.

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