September 2008 Notes *Chem. Pharm. Bull.* **56**(9) 1335—1337 (2008) 1335

Emulsion Preparation Using *β*-Cyclodextrin and Its Derivatives Acting as an Emulsifier

Motoki INOUE, Kaname HASHIZAKI, Hiroyuki TAGUCHI, and Yoshihiro SAITO*

College of Pharmacy, Nihon University; 7–7–1 Narashinodai, Funabashi, Chiba 274–8555, Japan.

Received April 7, 2008; accepted June 12, 2008; published online June 16, 2008

The preparation and formation mechanism of *n*-hexadecane/water emulsions using natural *β*-cyclodextrin (*β*-CD) and chemically modified *β*-CDs (triacylated *β*-cyclodextrins) as an emulsifier were investigated. The stable water/oil (W/O) emulsion was formed using tripropanoyl-*β*-CD (TP-*β*-CD). From observation using the contact angle (θ_{ow}) of precipitates derived from CD, it was clarified that oil/water (O/W) emulsion at $\theta_{ow}<90°$ and (W/O) emulsion at $\theta_{ow}>90°$ are formed when the composition of each oil and water was mixed with natural *β*-CD or triacylated *β*-CDs.

Key words cyclodextrin; phase diagram; oil/water emulsion; water/oil emulsion; Pickering emulsion; contact angle

Cyclodextrins (CDs) have a hydrophobic cavity and form an inclusion complex with a hydrophobic guest molecule; as a result, these have found a number of applications in the pharmaceutical field.[1)] We have recently reported the preparation and formation mechanism of emulsions using natural CDs as an emulsifier.[2,3)] In these studies, it was suggested that the formation of a dense film at the oil–water interface and a three-dimensional structural network in the continuous phase by precipitated complexes derived from CD are necessary for the formation of a stable emulsion. Consequently, we concluded that the formed emulsions are a kind of Pickering emulsion, which is stabilized by solid particles. Furthermore, we clarified that the prepared emulsion using natural CDs forms the oil/water (O/W) type only because the contact angle (θ_{ow}), which the precipitate derived from CD makes with the oil–water interface, is less than 90°. In consideration of the above results, it is thought that water/oil (W/O) emulsion must be formed at $\theta_{ow}>90°$. However, there is no report in the literature on the preparation of W/O emulsion using CDs as an emulsifier.

In this paper, we attempted to prepare W/O emulsion using triacylated *β*-CDs, which are hydrophobic compounds.

Experimental

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

Preparation of Triacylated *β*-CDs Triacylated *β*-CDs (triacetyl-*β*-CD (TA-*β*-CD), tripropanoyl-*β*-CD (TP-*β*-CD), and tributanoyl-*β*-CD (TB-*β*-CD)) were prepared using the corresponding acid anhydrides in pyridine.[4)] NMR spectra were measured on a JEOL JNM-LA400 spectrometer (Tokyo, Japan) operating at 400 and 100 MHz for protons and carbons, respectively. Chemical shifts (δ) are given in ppm relative to TMS as an external standard. FAB-MS spectra were measured in positive ion mode by a JEOL GC-mate (Tokyo, Japan). Chart 1 shows the chemical structures of triacylated *β*-CDs. NMR spectroscopic data and some analytical data of triacylated *β*-CDs are described below.

TA-*β*-CD: ^{1}H-NMR (DMSO) δ: 2.02—2.10 (9H, m), 3.84—3.89 (1H, t), 4.10—4.13 (1H, q), 4.25—4.29 (1H, q), 4.42—4.44 (1H, d), 4.74—4.77 (1H, q), 5.09 (1H, d), 5.21—5.26 (1H, t). ^{13}C-NMR (DMSO) δ: 20.49, 62.32, 69.36, 69.92, 69.99, 76.52, 96.55, 169.28, 169.99, 170.03. IR (KBr) cm^{-1}: 1750, 1380, 1240, 1040. FAB-MS *m/z*: 2017.8. *Anal.* Calcd for $C_{84}H_{112}O_{56}$: C, 50.00; H, 5.59. Found: C, 49.62; H, 5.71.

TP-*β*-CD: ^{1}H-NMR (DMSO) δ: 1.00—1.07 (9H, m), 2.21—2.40 (6H, m), 3.83—3.87 (1H, t), 4.10—4.13 (1H, q), 4.25—4.28 (1H, d), 4.39—4.42 (1H, d), 4.72—4.75 (1H, t), 5.05—5.06 (1H, d), 5.22—5.26 (1H, t). ^{13}C-NMR (DMSO) δ: 8.61, 26.60, 62.19, 69.31, 69.75, 70.09, 76.02, 96.27, 172.42, 173.23, 173.93. IR (KBr) cm^{-1}: 1750, 1470, 1360, 1180, 1050. FAB-MS *m/z*: 2312. *Anal.* Calcd for $C_{105}H_{154}O_{56}$: C, 54.54; H, 6.71. Found: C, 54.59; H, 6.83.

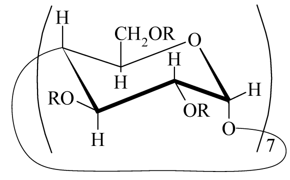
TB-*β*-CD: ^{1}H-NMR (DMSO) δ: 0.83—0.90 (9H, m), 1.49—1.56 (6H, m), 2.28—2.31 (6H, m), 3.84—3.85 (1H, t), 4.07—4.09 (1H, q), 4.24—4.26 (1H, d), 4.36—4.39 (1H, d), 4.67—4.70 (1H, t), 5.02—5.03 (1H, d), 5.20—5.25 (1H, t). ^{13}C-NMR (DMSO) δ: 13.20, 17.48—35.13, 62.10, 69.32, 69.69, 70.17, 76.05, 96.22, 171.38, 172.25, 172.30. IR (KBr) cm^{-1}: 1750, 1180, 1050. FAB-MS *m/z*: 2606.8. *Anal.* Calcd for $C_{126}H_{196}O_{56}$: C, 58.05; H, 7.58. Found: C, 58.01; H, 7.75.

Preparation of Emulsion and Phase Diagram The composition with different levels of CD, oil, 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 oil/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.[5)]

Contact Angle The contact angle measurement was the same as described previously.[3)] Briefly, the emulsion was centrifuged and the obtained precipitate 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 microcamera. The contact angle was then determined by an image analysis system (Visual Scalar MCP-550, Moritex Co., Tokyo, Japan). The measurements were repeated three times.

Results and Discussion

Phase diagrams of the ternary components of oil/CD/water systems were investigated. The oil phase used *n*-hexadecane, which formed the most stable emulsion in *n*-alkanes used as the oil phase in a previous paper.[3)] Figure 1 shows phase dia-



β-CD : R=–H
TA-β-CD : R=–$COCH_3$
TP-β-CD : R=–COC_2H_5
TB-β-CD : R=–COC_3H_7

Chart 1. Chemical Structures of Triacylated-*β*-CDs

* To whom correspondence should be addressed. e-mail: ysaito@pha.nihon-u.ac.jp

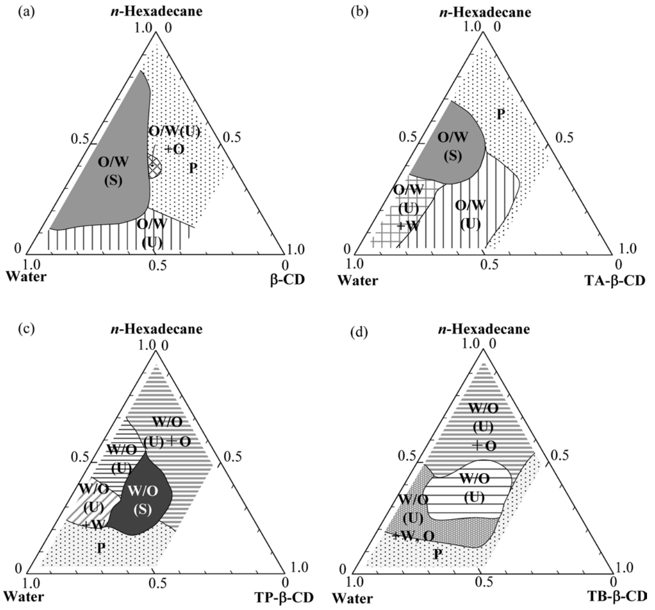


Fig. 1. Phase Diagrams of *n*-Hexadecane/CD/Water Systems at 25 °C.

(a) *n*-Hexadecane/β-CD/water system, (b) *n*-hexadecane/TA-β-CD/water system, (c) *n*-hexadecane/TP-β-CD/water system, and (d) *n*-hexadecane/TB-β-CD/water system.

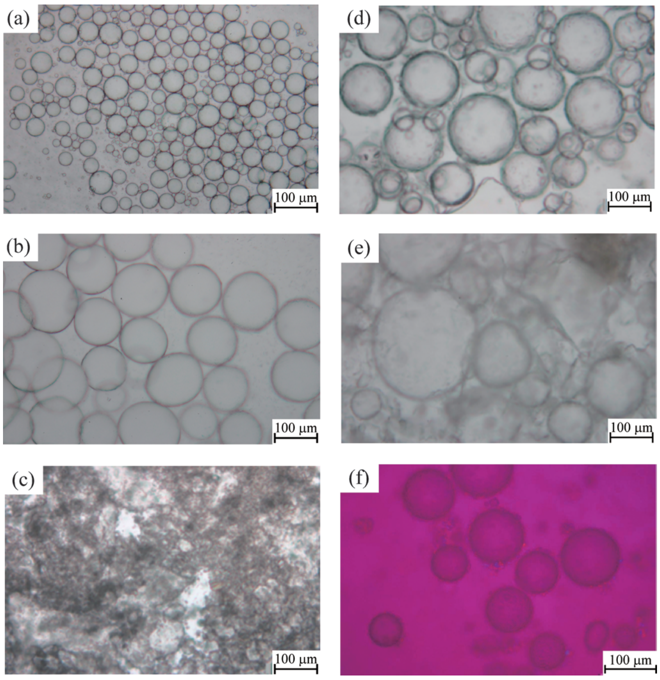


Fig. 2. Microphotographs of *n*-Hexadecane/CD/Water Systems

(a) Stable O/W emulsion phase, (b) unstable O/W emulsion phase, (c) precipitated phase, (d) stable W/O emulsion phase, (e) unstable W/O emulsion phase, and (f) stable W/O emulsion phase taken under cross nicol polarization. (a), (b) and (c) are emulsified using TA-β-CD, and (d), (e) and (f) are emulsified using TP-β-CD.

grams of the ternary components of *n*-hexadecane/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, 'W/O (S)' is a stable W/O emulsion, 'W/O (U)' is an unstable W/O emulsion, 'O' is the separated oil phase, 'W' is the separated water phase, and 'P' is the precipitate phase.

Figure 2 shows the microphotographs and the microphotograph under crossed nicol polarization for each phase in *n*-hexadecane/CD/water systems. Stable O/W emulsions were formed using β-CD and TA-β-CD, while the stable W/O emulsion was formed using TP-β-CD. For TB-β-CD, only the unstable W/O emulsion was formed. In the low volume fraction of the dispersed phase in the system, the emulsion was liable to be unstable regardless of the kind of β-CDs. In addition, in the low volume fraction of the continuous phase and in the high volume fraction of CD in the system, the formation of precipitates was confirmed in all systems. In addition, the phase inversion in the emulsion by the change in the volume fraction of oil and water did not occur in all systems. The surface state of W/O emulsion droplets was investigated using polarized light microscopy. From the photograph, it was clear that the precipitates contributed to the formation of the emulsion because polarized light originating from precipitates was observed on the surface of droplets (see Fig. 2f). Consequently, it was thought that the formed W/O emulsion is a kind of Pickering emulsion, similar to the O/W emulsion previously reported.[2,3]

The type of Pickering emulsion is governed by the wettability of the solid particles at the oil–water interface[6,7]; 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, as shown

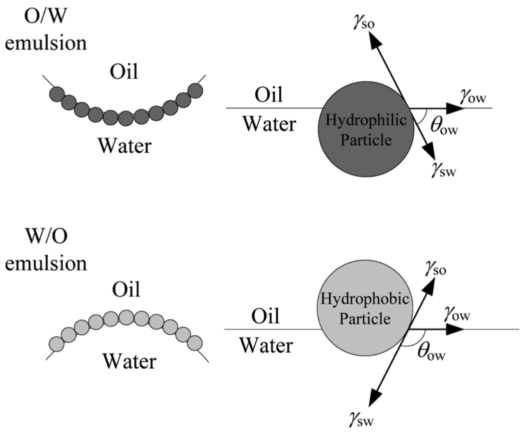


Fig. 3. Formation Mechanism of Pickering Emulsion

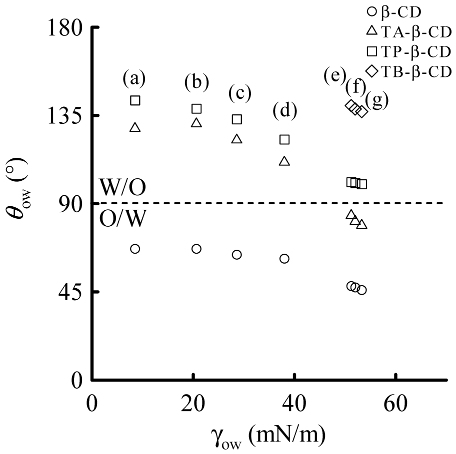


Fig. 4. Relationship between the Contact Angle (θ_{ow}) Which the Precipitate Makes with the Oil–Water Interface and Interfacial Tension (γ_{ow}) of Various Compounds Used as an Oil Phase at 25 °C

(a) *n*-Octanol, (b) methyl *n*-octanoate, (c) isopropyl myristate, (d) poly(dimethylsiloxane), (e) *n*-octane, (f) *n*-dodecane, and (g) *n*-hexadecane. The γ_{ow} values of various compounds are from refs. 9 and 10.

in Fig. 3. In addition, it is known that the θ_{ow} varies with the oil-water interfacial tension (γ_{ow}) of the compound used as the oil phase.[8] Figure 4 shows the correlation between the θ_{ow} which the precipitate makes with the interface and the γ_{ow} of various compounds. In the TB-β-CD, the θ_{ow} values in oils having a low γ_{ow} were not measured because the hydrophobicity of precipitates derived from TB-β-CD is very high. The θ_{ow} values increased with increasing hydrophobicity of CD and decreasing interfacial tension of the compound used as the oil phase. Furthermore, O/W emulsion at $\theta_{\mathrm{ow}}<90°$ and W/O emulsion at $\theta_{\mathrm{ow}}>90°$ were formed when the composition of each compound (oil) and water was mixed with natural β-CD or triacylated β-CDs. In the TA-β-CD, it is particularly interesting that W/O emulsion is formed using oils having a low γ_{ow}, while O/W emulsion is formed using oils having a high γ_{ow}.

In conclusion, the present findings suggest that both O/W and W/O emulsions can be prepared using appropriate β-CDs and oils.

Acknowledgment 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.

References

1) Frömming K.-H., Szejtli J., "Cyclodextrins in Pharmacy," Kluwer Academic Publishers, Dordrecht, 1994.
2) Hashizaki K., Kageyama T., Inoue M., Taguchi H., Ueda H., Saito Y., *Chem. Pharm. Bull.*, **55**, 1620—1625 (2007).
3) Inoue M., Hashizaki K., Taguchi H., Saito Y., *Chem. Pharm. Bull.*, **56**, 668—671 (2008).
4) Hirayama F., Yamanaka M., Horikawa T., Uekama K., *Chem. Pharm. Bull.*, **43**, 130—136 (1995).
5) Martin A., Swarbrick J., Cammarata A., "Physical Pharmacy," 3rd ed., Lea & Febiger, Philadelphia, 1983, pp. 553—554.
6) Schulman J. H., Leja J., *Trans. Faraday Soc.*, **50**, 598—605 (1954).
7) Binks B. P., Lumsdon S. O., *Langmuir*, **16**, 8622—8631 (2000).
8) Binks B. P., Lumsdon S. O., *Langmuir*, **16**, 2539—2547 (2000).
9) "Kagaku Binran Kisohen II," ed. by The Chemical Society of Japan, Maruzen, Tokyo, 1988, pp. 88—89.
10) Binks B. P., Lumsdon S. O., *Phys. Chem. Chem. Phys.*, **2**, 2959—2967 (2000).