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Structural and physical characterization of octenylsuccinyl β-cyclodextrin

Jeong-Kwan Choi[a], Tomasz Girek[b], Dong-Hoon Shin[c], Seung-Taik Lim[c,*]

[a]*Food Research Center, SAMYANG GENEX Corporation, 285, Gajwa-dong, Seo-ku, Inchon 404-205, South Korea*
[b]*Institute of Chemistry, Pedagogical University, Armii Krajowej Ave. 13/15, 42-200 Czestochowa, Poland*
[c]*Graduate School of Biotechnology, Korea University, 1,5-Ka, Anam-dong, Sungbuk-ku, Seoul 136-701, South Korea*

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Abstract

Beta-cyclodextrin (β-CD) was partially substituted by 2-octen-1-ylsuccinic anhydride, via its oxyanion prepared from a reaction with NaH in *N*,*N*-dimethyl formamide. ^{13}C and ^{1}H NMR spectra revealed that the substitution occurred mainly on C-2 hydroxyl group of glycosyl units. In the reaction mixture, three major derivatives with different average degree of substitution (DS) (0.723, 0.493, and 0.178) were detected by thin layer chromatography. The substitution increased as addition of the anhydride and reaction time increased, but decreased with reaction temperature. The octenylsuccinyl β-CD was highly soluble not only in water (>40%, w/w) but also in aqueous solutions of alcohol (>30% solubility in 50% ethanol) and salt (>40% in 1% NaCl or $CaCl_2$). The solubility decreased with increasing acidity of the solution. In an acidic citrate buffer solution (pH 3 or 5), β-CD derivative of low DS (0.178) showed a higher solubility (>30%) than that of high DS (0.518, 1–4%). The octenylsuccinyl derivatives had raised abilities for complexation and dissolution for *trans*-retinol. Solubility of the retinol in water became 6.57 mg/ml with an octenylsuccinyl β-CD complexation (DS 0.518, 100 mg/ml). The β-CD derivatives could also act as emulsifiers, forming a stable emulsion with linoleic acid.

Keywords: β-Cyclodextrin; Octenylsuccinylation; Solubility; Retinol; Emulsification

1. Introduction

Cyclodextrin (CD) is a cyclic oligosaccharide composed of α-1,4 linked D-glucopyranosyl units. It is normally produced by the action of cyclodextrin glucosyltransferase on starch (Szejtli, 1982). β-cyclodextrin contains 7 glycosyl units and has the highest production yields, but its lower water solubility limits its industrial use (Szejtli, 1988).

This low solubility is due to hydrogen bonds between the secondary hydroxyl groups of the adjacent glycosyl units. In order to increase the solubility of β-CD, modification with hydrophilic groups such as carboxyl or phosphoryl groups has been used (Lee & Lim, 1998; Roehri-Stoeckel, Dangles & Brouillard, 1997). Methylated, hydroxyalkylated, acetylated, or branched derivatives were also studied to improve water solubility and inclusion ability of β-CD.

The primary hydroxyl group (OH-6) of a glycosyl unit in β-CD is basic, whereas the secondary hydroxyl groups are acidic (Khan, Forgo, Stine & D'Souza, 1998). In a weak basic solution, a low reactive and non-complexing electrophile is substituted mainly on OH-6. In a strong basic condition, however, a proton is removed from the hydroxyl group on C-2 prior to substitution, and the non-complexing electrophile reacts on the β-CD oxyanion, to produce a C-2 substituted derivative. Using the oxyanion an intermediate formed by a reaction with NaH, 2-*O*-(4-methylamino-3-nitro)benzyl-β-CD and maleic estered polymer have been prepared (Girek, Shin & Lim, 2000; Rong & D'Souza, 1990). The oxyanion could also be made with NaOH in dimethyl sulphoxide (Ishimaru, Masuda & Iida, 1997). β-CD epoxide was used as a similar intermediate for the substitution on secondary hydroxyl group, and reaction products from the epoxide were similar to those prepared from the oxyanion (Van Dienst, Snellink, Von Piekartz, Grote Gansey, Venema, Feiters et al., 1995). After protection of the primary hydroxyl groups, heptakis(2-tosyl)-β-CD was made by NaH deprotonation of OH-2 followed by the reaction with tosyl chloride, and it was further reacted to make manno-(2,3-epoxy)-β-cyclodextrin (Khan, Barton & D'Souza, 1996).

The molecular conformation of β-CD is torus-like with a hydrophobic inner space. This structural characteristic provides a unique ability for complex formation with various hydrophobic guest compounds. The physical properties of the guest compounds are changed by the

* Corresponding author. Tel: +82-2-3290-3435; fax: +82-2927-5201.
E-mail address: limst@korea.ac.kr (S.-T. Lim).

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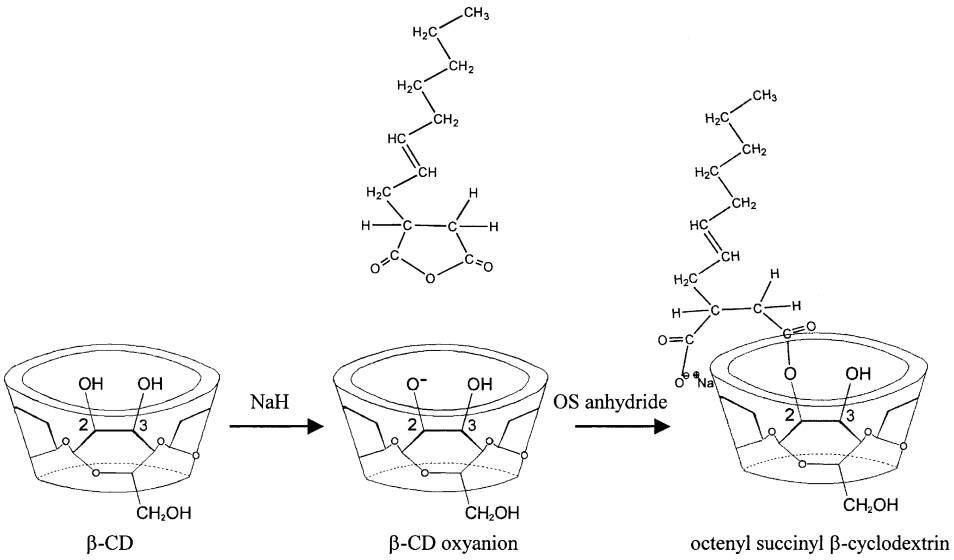

Fig. 1. Schematic presentation for octenylsuccinylation of β-cyclodextrin.

complexation. For example, a CD-complexed hydrophobic guest compound can become readily soluble (Uekama, Hirayama & Irie, 1998). Vitamin A, which is susceptible to inactivation (Budavari, O'Neil, Smith, Heckelman & Kinneary, 1996), can be protected in complexation with native or substituted β-CD. Heptakis(2,6-*O*-dimethyl)-β-CD could bind all *trans*-retinol, retinal, and retinoic acid providing substantially raised stability. It has been reported that the substituted methyl groups enhanced the complexing ability of β-CD (Guo, Ren, Fang & Liu, 1995). The β-CD derivatives have been used in various applications including cosmetics, foods, enzyme mimics, drug delivery systems, chromatography resins, etc (Croft & Bartsch, 1983).

Octenylsuccinylation, enhances the surface activity of a starch so that it can be used as an oil extender or an emulsifier in foods (Cho, Lim, Park, Hwang & Lim, 1999). These properties result from the amphiphilic nature of the octenylsuccinyl group, which arise from the hydrophobic octenyl chain and the hydrophilic succinate group. In this study, β-CD was chemically substituted by the octenylsuccinate group through a β-CD oxyanion intermediate, and its solubility and emulsifying ability were examined.

2. Experimental

2.1. Materials

Commercial crystalline β-cyclodextrin was obtained from the DAESANG Company (Seoul, Korea) and dried for 4 h in a vacuum oven at 100°C prior to use. 2-Octen-1-ylsuccinic anhydride (97% purity, mixture of *cis* and *trans*-form) and sodium hydride (NaH) were purchased from the Aldrich Chemical Company (Milwaukee, WI). *N*,*N*-Dimethyl formamide (DMF) was purchased from the Showa Chemical Company (Tokyo, Japan), and purified by vacuum distillation (Armarego & Perrin, 1996). All *trans*-retinol and linoleic acid (*cis*-9,*cis*-12-octadecadienoic acid, approx. 60%, free acid form) were purchased from the Sigma Chemical Company (St. Louis, MO).

2.2. Preparation of octenylsuccinyl β-CD

Fig. 1 shows the scheme for the preparation of octenylsuccinyl β-CD (OS-β-CD). The dried cyclodextrin (5 g, 4.4 mmol) was dissolved in purified DMF (45 ml) by

Table 1
Reaction conditions for octenylsuccinylation of β-CD

OS-β-CDs	Molar ratio of β-CD/NaH/OS anhydride	Reaction temperature and time		Degree of substitution
		Deprotonation	Octenylsuccinylation	
A	1:3:3	25°C, 6 h	25°C, 3 h	0.518
B	1:1:1	25°C, 6 h	25°C, 3 h	0.178
C	1:1:1	25°C, 3 h	25°C, 3 h	0.179
D	1:1:1	25°C, 1 h	25°C, 3 h	0.150
E	1:1:1	25°C, 6 h	100°C, 3 h	0.147
F	1:1:1	100°C, 3 h	100°C, 3 h	0.115
G	1:1:1	100°C, 1 h	100°C, 3 h	0.123

stirring for 4 h at ambient temperature. After NaH (0.106 g, equivalent molar ratio to β-CD) was added, the mixture was stirred in a sealed flask for 6 h to make β-CD oxyanion (Rong & D'Souza, 1990). An aliquot of octenylsuccinic anhydride (OS anhydride) (0.955 ml, equivalent molar ratio to β-CD) was added slowly to the mixture, and stirring was continued for 3 h. The reaction was terminated by adding water (10 ml), and then the mixture was concentrated to syrup by using a rotary vacuum evaporator. The vacuum evaporation with water was repeated three times to remove the residual solvent. The amorphous precipitate was obtained with acetone (400 ml), and then vacuum-dried. Alternatively, the molar ratio of β-CD/NaH/OS anhydride, the reaction temperature, and time were changed to 1:3:3, 100°C, and 6 h, respectively (Table 1).

2.3. Isolation of octenylsuccinyl β-CD

The octenylsuccinyl derivatives were isolated using flash chromatography packed with a silica gel (Silica Gel 60, Merck, Darmstadt, Germany) (Still, Khan & Mitra, 1978). An eluent mixed with ethyl acetate, ethanol and water (6:3:2 by volume) was used, and the isolated derivatives were characterized by thin layer chromatography (TLC, Silica Gel $60F_{254}$, Merck, Darmstadt, Germany) with the same solution used in flash chromatography.

2.4. NMR spectroscopy

β-CD derivatives were dissolved in Me_2SO-d_6, (approx. 20 mg in 0.6 ml), and Me_4Si was added as an internal reference. ^1H and ^{13}C NMR spectra were obtained at 30°C with an AMX-500 NMR spectrometer (Bruker Instrument, Rheinstetten, Germany), whereas the heteronuclear chemical shift correlation spectrum was recorded using a DMX-600 NMR spectrometer.

The degree of substitution for octenylsuccinylation was calculated from the ratio of the areas of the two integrated peaks for terminal methyl protons of the octenyl group and anomeric protons of β-CD on the ^1H NMR spectrum, according to the following equation,

$$\text{Degree of substitution} = \frac{\text{peak area for methyl protons in octenyl group}/3}{\text{peak area for anomeric protons of CD}}$$

2.5. Molecular weight analysis

The weight-average absolute molecular weight (M_w) was determined using a multi-angle laser light scattering detector (MALLS, DAWN DSP-F, 632.8 nm, Wyatt Technology, Santa Barbara, CA) and a refractive index detector (RI-71, 25°C, Shodex, Tokyo, Japan) connected to a size exclusion high performance liquid column chromatography (HPLC, TSK 3000). The dn/dc value (increment of refractive index by concentration) of the β-CD derivatives was measured by an interferometric refractometer (OPTILAB DSP, Wyatt Technology, Santa Barbara, CA).

The HPLC system consisted of a pump (P2000, Spectra System, San Jose, CA), an injection valve (model 7072, Rheodyne, Cotati, CA), and TSK-Gel G3000PWXL column (TosoHaas Corp., Tokyo, Japan). Mobile phase was 0.15 M $NaNO_3$ containing 0.02% NaN_3. The octenylsuccinyl β-CD was dissolved in the mobile phase, and then the solution was filtered through a 1.0 μm cellulose nitrate membrane filter before injection. The injection volume was 500 μl, and ASTRA 4.50 software was used for calculation of the M_w. The column temperature and the flow rate were 25°C and 0.5 ml/min, respectively.

2.6. Solubility of β-CD derivatives

Aqueous solutions containing different concentrations of ethanol (0, 25, 50, 75, and 99%, v/v), 1% NaCl solution, 1% $CaCl_2$ solution, and 0.5 M citrate buffer solutions (pH 3 and 5) were tested for the solubility of the β-CD derivatives. To exclude the effects of citrate salt, aqueous dispersion of the derivatives adjusted to pH 3 or 1 by adding 0.1 M HCl was also tested.

The solubility was measured by saturating β-CD derivative in solution (1 ml) followed by vigorous stirring at 25°C for 4 h. The precipitate was removed by centrifugation (15,300 g, 10 min), and then the solids (dissolved β-CD) in the supernatant were measured after drying to a constant weight at 105°C.

2.7. Dissolution of all trans-retinol

Excess retinol was added to the aqueous β-CD solutions at different concentrations (0–100 mg/ml) which were adjusted to pH 6.5 ± 0.5. The retinol solution was treated by ultra-sonication for 30 s, and then vigorously stirred at 25°C for 2 h. The solution was centrifuged (15,300 g, 10 min), and the retinol content in the supernatant was measured from the absorbance at 325 nm. A calibration curve was measured using standard retinal solutions (0, 20, 40, 60, 80 ppm) in 99% ethanol.

2.8. Emulsifying ability

Oil in Water (O/W) emulsion was prepared with linoleic acid and β-CD solution. An aqueous solution of β-CD (350 mg in 70 ml) was emulsified with linoleic acid (40 ml) at 10,000 rpm for 5 mins with a homogenizer (Nihonseiki Kaisha Ltd., Tokyo, Japan). Fat globules in the emulsion were observed using a light microscope (Olympus Optical Co., Ltd., Tokyo, Japan), and the emulsion was transferred to a measuring cylinder (100 ml) for the observation of separation during storage at ambient temperature for 24 h (Dickinson, Ritzoulis & Povey, 1999).

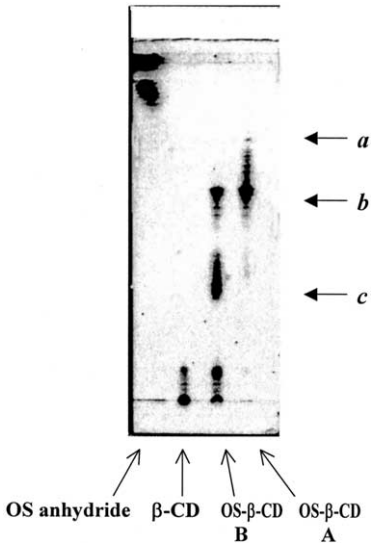

Fig. 2. Thin layer chromatograms of octenylsuccinic anhydride, β-CDs, and OS-β-CD (A and B).

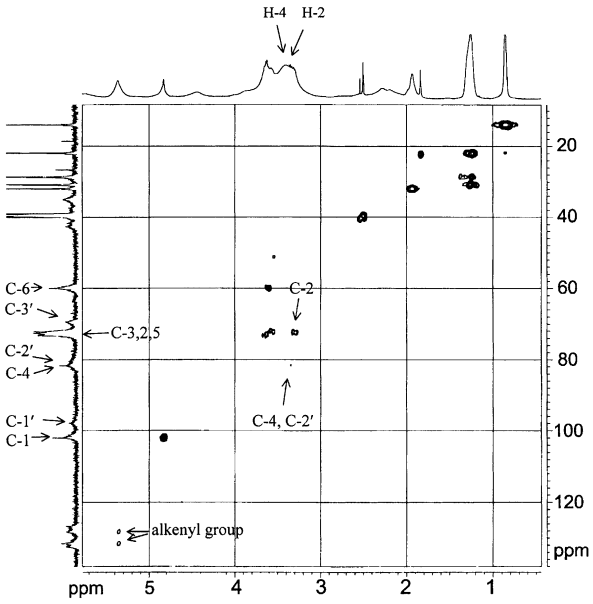

Fig. 3. Heteronuclear chemical shift correlation spectrum of OS-β-CD.

3. Results and discussion

3.1. Reaction tendency

Table 1 shows the degree of substitution of the β-CD derivatives prepared under different reaction conditions. Under conditions B, C and D, the degree of substitution of the reaction product increased only slightly as the deprotonation time changed from 1 to 6 h. Therefore, the reaction with NaH occurred mostly within 3 h. The β-CD product under condition E had a lower degree of substitution than that under B. Similarly, the products under conditions F and G showed lower degrees of substitution than those prepared at the lower temperature (25°C, C and D). The product prepared at higher temperature for longer time (F) than that under condition G, also showed a lower degree of substitution.

The β-CD derivative prepared with excess of the anhydride reagent (condition A), in comparison with that under condition B, showed a higher degree of substitution. Therefore, the octenylsuccinylation of β-CD could be increased with the increase of the anhydride amount and reaction period, but reduced as the reaction temperature increased.

3.2. Thin layer chromatogram

Fig. 2 shows a thin layer chromatogram of the reaction mixtures for octenylsuccinylation at different ratios of β-CD, NaH and OS anhydride (A and B in Table 1). The β-CD products (OS-β-CD) appeared containing three major fractions on the chromatogram (a, b and c). The R_f value of these fractions increased as the anhydride addition increased. Under the reaction condition B (Table 1), a significant amount of unreacted β-CD remained in the reaction mixture, whereas most β-CD reacted under condition A. Fraction a (R_f 0.71) was found exclusively in the reaction mixture A, and fraction c (R_f 0.29) was found only in the reaction mixture B. However, fraction b (R_f 0.59) was found in both reaction mixtures. These fractions were isolated from the chromatogram for structural analysis using NMR spectroscopy.

3.3. NMR spectroscopy

Fig. 3 shows a heteronuclear chemical shift correlation spectrum for the octenylsuccinyl β-CD fraction b isolated from the reaction mixture A on the thin layer chromatogram. ^{13}C-signals of native β-CD were found, in ppm, at 101.91 (C-1), 81.51 (C-4), 73.01 (C-3), 72.36 (C-2), 71.98 (C-5), and 59.90 (C-6) in agreement with the values reported in literatures (Christofides & Davies, 1983; Hao, Tong, Zhang & Gao, 1995; Rong & D'Souza, 1990; Schneider, Hacket & Rüdiger, 1998). The double bond in the octenyl chain was identified by the signals in a range from 125 to 135 ppm. In the spectrum, signals for C-1 and C-3 were shifted upfield (97.72 and 69.29 ppm, respectively for C-1′ and C-3′) due to the shielding effect from the substituent, indicating that the substitution occurred on C-2 (Hao et al., 1995; Ishimaru et al., 1997; Rong & D'Souza, 1990; Ueno & Breslow, 1982). However, the substituted C-2′ was not clearly shown on the spectrum. The C-2′ signal was deshielded by the substituent and moved downfield, and was found to overlap the C-4 signal. The C-2′ signal was located at about 81.0 ppm, which was shifted by 8.64 ppm by the substitution.

3.4. Degree of substitution

Fig. 4 shows ^{1}H NMR spectra for alkenyl group, anomeric carbon (H-1), and three hydroxyl groups (OH-2, -3 and -6)

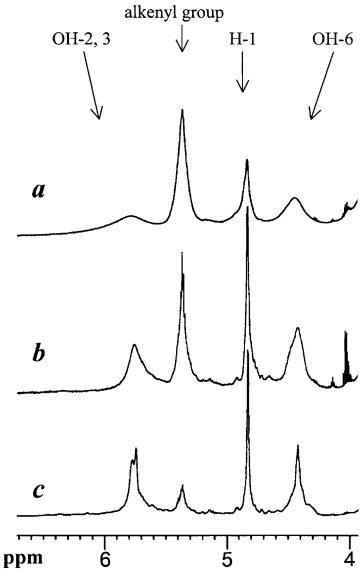

Fig. 4. ^{1}H NMR spectra of OS-β-CD fractions (a, b, and c).

of the glycosyl units in β-CD, all of which appeared at 4–6 ppm. For the fraction a on thin layer chromatogram, peak intensity for OH-2 and -3 was least, but that of the alkenyl double bond was highest. There was almost no difference in peak intensity ratio between primary proton (OH-6) and H-1 among the three fractions, whereas the ratio between the secondary protons (OH-2, 3) and H-1 was significantly different among the fractions. This result proved that no or insignificant otenylsuccinylation occurred on the primary hydroxyl group, but the reaction occurred mainly or exclusively on the secondary hydroxyl groups (OH-2), based on the previous heteronuclear spectrum.

The degree of substitution per glycosyl unit and the number of substituents per β-CD were calculated from the ratio of the integrated peak area between the methyl protons (0.7–1.0 ppm) of the alkenyl group and the anomeric proton. Fraction a, b and c had DS values of 0.723, 0.493, and 0.178, respectively. Converting these values to the average number of substituents per β-CD, about five octenylsuccinyl groups were substituted on a β-CD molecule in the fraction a. In the same way, three or four groups were substituted in the fraction b, and one group in the fraction c. But, these calculations were based on an assumption that all the substituents were linked to β-CD by only one-ester bond.

3.5. Molecular weight

The d*n*/d*c* values of native β-CD and the β-CD reaction mixtures from conditions A and B, were 0.139, 0.145, and 0.137 (ml/g), respectively. The significantly higher value for reaction mixture A might suggest that the structure of the β-CD molecules in the reaction product was quite different from native β-CD or the product from condition B. This was probably caused by the high degree of substitution of fraction.

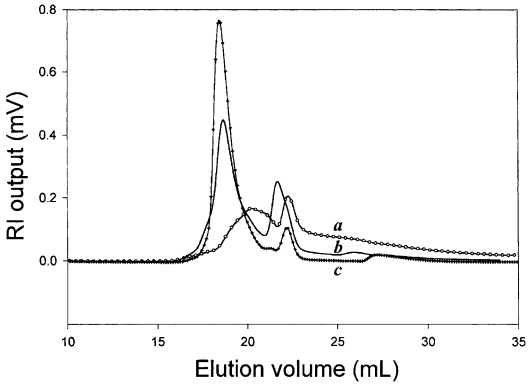

Fig. 5. HPLC-RI-MALLS chromatograms of OS-β-CD fractions (a, b, and c).

Three octenylsuccinyl β-CD fractions (a, b, c) isolated from thin layer chromatogram were analyzed by size exclusion chromatography (SEC), and the RI chromatograms are shown in Fig. 5. It was found that the chromatogram of each fraction consisted of two major peaks. Fraction a isolated from TLC showed two peaks with weight-average molecular weight values (M_w) of 5500 and 3800. The M_w values of two components in fraction b were 3300 and 2200, and those in fraction c were 2800 and 2600, from the calculation based on RI outputs and light scattering intensities.

The calculated M_w values did not exactly fit the number of octenylsuccinate groups per β-CD determined by NMR. For example, if the β-CD derivatives in fraction c have on average one substituent per β-CD, the molecular weight (M_w) should be 1345 which is about half the M_w measured by SEC system. The discrepancy between NMR and SEC analyses could be due to the relatively low resolution ability of the light scattering detector for small analytes. Assuming the SEC data is correct, two possible explanations for the conformation of β-CD derivatives could be suggested: dimerization of two β-CDs and β-CD/β-CD complex formation. It is likely that the hydrophobic chain of the octenyl group attached to β-CD form an inclusion complex with a center cavity of other β-CD molecules. In addition, dimer formation through an ester linkage upon a succinate could be possible because the reaction underwent in anhydrous medium. The significant difference in d*n*/d*c* among the reaction products indicates that these new structures could be present. Further structural analysis will be followed for clear identification.

3.6. Solubility in ethanol and salt solutions

Solubility of the octenylsuccinyl β-CD prepared in different reaction conditions (A and B, Table 1) was tested in aqueous ethanol solutions. Unmodified β-CD exhibited a gradual decrease in solubility as the ethanol concentration increased from 0 to 99% (v/v) (data not shown). But the solubility in 25% ethanol solution was slightly higher than that in pure water. The higher solubility was caused by

Table 2
Solubility (%, w/w) of OS-β-CDs in citrate buffer (pH 5 and 3) and dilute HCl solutions (pH 3 and 1) at 25°C

	pH 5 Citrate	pH 3 Citrate	pH 3 HCl	pH 1 HCl
OS-β-CD A	1.89	2.75	3.89	1.09
OS-β-CD B	> 30[a]	> 30	24.75	17.33
β-CD	4.50	9.65	2.63	1.49

[a] Solubility is over 30%.

formation of an inclusion complex between β-CD and alcohol (Szejtli, 1988).

The octenylsuccinyl β-CDs displayed much higher solubilities in aqueous ethanol solutions than unreacted β-CD. The β-CD derivative prepared under reaction condition A showed an exceptionally high solubility (over 40%, w/w) even in 75% (v/v) ethanol solution. Preparation of the saturated solution in alcohol was difficult because of its high solubility. β-CD derivative prepared under reaction condition B, however, showed a relatively low solubility (3.90%) in 75% ethanol, although it was highly soluble (over 40%) in 25% ethanol. The differences in alcohol solubility between the two β-CD products might be caused by differences in hydrophobicity due to the octenyl chain in the substituent as well as the conformation (dimer or complex).

The β-CD derivatives also were highly soluble in dilute salt solutions (data not shown). The solubility of the β-CD derivatives, regardless of the reaction condition, was over 40% in 1% NaCl or $CaCl_2$ solution, whereas the solubility of unreacted β-CD was about 2.4%. The substitution caused the breakage of hydrogen bonds between the secondary hydroxyl groups of the β-CD ring, and thus β-CD became more soluble in aqueous salt solutions. The same theory has been explained in a study on partial methylation of β-CD (Szejtli, 1982).

3.7. Solubility at low pH

Table 2 shows the solubility of native and octenylsuccinyl β-CDs (OS-β-CDs) in several acidic solutions. In mild acidic solutions (pH 5 and 3) buffered with 0.5 M citrate, the β-CD derivative under condition A (OS-β-CD A) showed a very low solubility, even lower than that of unreacted β-CD, whereas that under condition B showed a solubility over 30%.

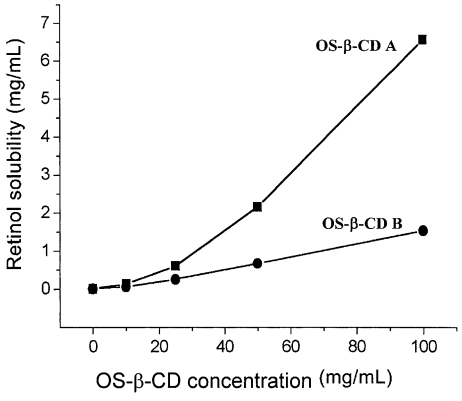

Fig. 6. Higuchi phase solubility diagram of all *trans*-retinol in aqueous OS-β-CD solutions.

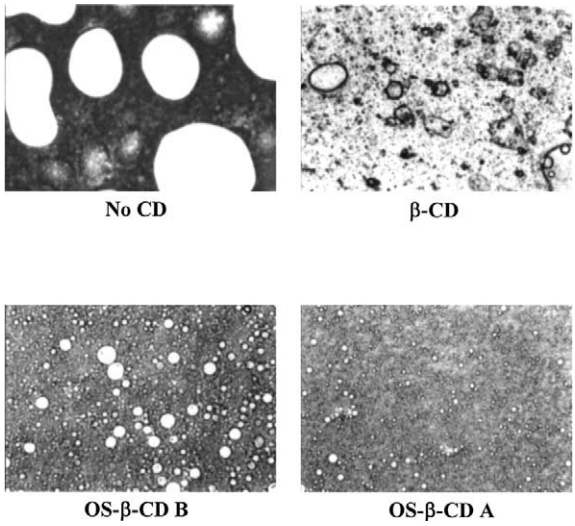

Fig. 7. Photomicrographs of linoleic acid-in-water emulsions with or without OS-β-CDs (0.5%).

Native β-CD had a higher solubility in citrate buffer than in dilute HCl, which was assumed to be from inclusion complex formation between β-CD and citrate. The complex formation with citrate might also occur in the case of β-CD derivative under reaction condition B (OS-β-CD B), because it showed a higher solubility in the buffer solutions than in dilute HCl at same pH. The acid dissociation constants (K_1 and K_2) of succinic acid are 6.21×10^{-5} and 2.32×10^{-6}, respectively (Skoog, West & Holler, 1994). Assuming that octenylsuccinic acid had similar dissociation constants, the octenylsuccinyl groups ester-linked on β-CD had free acidic form in a buffer solution below pH 5. The particular low solubility of OS-β-CD A was possibly related to the free acidic form of the octenylsuccinyl groups which make no contribution to ionic strength (Uekama et al., 1998). The OS-β-CD B was less substituted than OS-β-CD A, and thus the susceptibility to acidic solution was expected to be lower. The low acid solubility of OS-β-CD A might be advantageous when used as a drug carrier since dissolution could be controlled by pH.

3.8. Phase solubility of retinol

Fig. 6 shows an increase in retinol solubility in water by using the β-CD derivatives. The OS-β-CD A, which had a high degree of substitution, exhibited a high efficiency in dissolving retinol, as shown in the A_P-type Higuchi phase solubility diagram (Higuchi & Connors, 1965). It shows that more than one OS-β-CD molecule was required to a complex with a retinol molecule. At the highest OS-β-CD A concentration (100 mg/ml), the solubility of retinol was

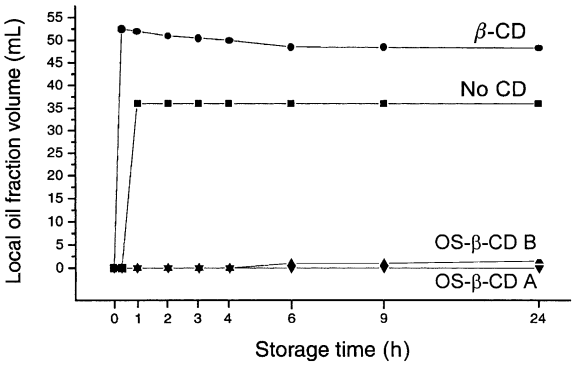

Fig. 8. Oil separation from the emulsions during a storage at ambient temperature.

0.66% (6.6 mg/ml). Assuming that all OS-β-CD A and retinol existed in complex form, the result indicates that on average 2.45 molecules of OS-β-CD A per retinol molecule were required to form an inclusion complex.

3.9. Emulsion properties

Fig. 7 shows photomicrographs of oil globules (linoleic acid) in the emulsions prepared with unreacted and substituted β-CDs. The size of the oil globules depended on the type of β-CD. Without β-CD, the oil globules were very large, and coagulated readily into separate phase. In the emulsion with unreacted β-CD also, the oil globules were large, but phase separation was somewhat retarded. Furthermore, insoluble β-CD particles were found in the emulsion on the photomicrograph. When the octenylsuccinyl β-CD was used, however, the oil globules became smaller, and the emulsion was more stable. The emulsion containing OS-β-CD A contained smaller globules, compared to the OS-β-CD B emulsion. The emulsion-forming ability might increase with the degree of substitution of octenylsuccinate groups because the octenylsuccinyl substituents enhanced the surface activity of β-CD.

The linoleic acid–water emulsions prepared with native and modified β-CDs were stored at an ambient temperature for 24 h, and periodically examined for emulsion stability. Separation of oil layer became significant on storage. Fig. 8 shows oil separation from the emulsions during the storage. The unreacted β-CD displayed a rapid separation of oil within 30 min after homogenization. But the separation was not as clear as pure water–oil emulsion (picture not shown). It was assumed that the β-CD in the solution partially complexed with the linoleic acid, and the complexed solids accumulated on the interface between oil and water. The emulsions containing OS-β-CD B or OS-β-CD A showed excellent stability without any phase separation up to 24 h of storage. The surface activity provided by the substituent might be useful when the β-CD is applied in various emulsions. Therefore, by the partial substitution with octenylsuccinyl group in β-CD, not only is the solubility in various aqueous solutions but also the emulsion stability with lipids substantially improved. These characteristic changes may enhance the utilization of β-CD in various industrial areas.

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