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Polymerization of β-cyclodextrin with maleic anhydride and structural characterization of the polymers

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Abstract

β-Cyclodextrin (β-CD) polymers were prepared by cross-linking β-CD with maleic anhydride (MA) in anhydrous *N*,*N*-dimethyl formamide (DMF) in the presence of NaH. The weight-average molecular weight (M_w) and the chemical structure of the polymers were determined using high performance size exclusion chromatography (HPSEC) with refractive index (RI) and multiangle laser-light scattering (MALLS) detectors, and ^{1}H NMR spectroscopy. The molecular weight of the polymer increased as the reaction temperature was raised. With a reaction of 4 h at 130°C at a molar ratio of 1:7:7 for β-CD:NaH:MA, the reaction products were water-insoluble, and contained major polymer fractions with M_w greater than 200 kDa. Smaller and water-soluble polymer fractions were produced at 100°C or lower reaction temperature. ^{1}H-NMR spectra revealed that the CD polymers contained both mono- and diesters of butenedioic acid, and diesters became prevalent as the reaction time and temperature were raised. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: Polymerization; β-Cyclodextrin; Maleic anhydride

1. Introduction

Cyclodextrins (CD) are cyclic oligomers composed of six, seven or eight anhydrous glucopyranosyl units (AGU) (known as α-, β-, γ-CD, respectively) linked together by α-1,4-linkages. In a CD molecule, all secondary hydroxyl groups at the C2 and C3 positions of the AGU protrude from the wide opening of the trunk shape of CD, whereas the primary hydroxyl groups at C6 are exposed from the opposite side (Bender & Komiyama, 1978). It is widely acknowledged that CD can form complexes with a wide variety of organic and inorganic substances in its hydrophobic cavity (Hinze, 1981; Szejtli, 1982, 1988). Because of this unique characteristic, CD has applications in the food, pharmaceutical and cosmetic industries.

When CD is polymerized, it becomes less soluble and more stable, and thus the derivative can be used to remove or isolate minor compounds from foods or other biological matrices (Shaw & Wilson, 1985; Shaw, Tatum & Wilson, 1984; Su & Yang, 1991). To polymerize CD, two common methods are normally used. In one way, CD molecules are attached as pendent groups on other polymer chains. Polymers with CD pendants are usually prepared by radical polymerization of the functional CD monomers such as acrylolyl cyclodextrin (CD-A) and *N*-acrylolyl-6-aminocaprocyclodextrin (CD-NAC) (Harada, Furue & Nozakura, 1975, 1976). These monomers may further copolymerize with other monomers such as acryl amide or acrylic acid. The second type of polymerization is to react CD molecules with bifunctional agents. The most common agent is epichlorohydrin, although other epoxy compounds such as ethylene glycol, bisepoxy(propyl) ether (Cserhati, Fenyvesi & Szejti, 1992) or butylene glycol bis(epoxypropyl)ether (Cserhati & Forgacs, 1994) have been used. These nucleophilic substitution reactions with bifunctional agents usually occur in strong alkaline conditions in order to deprotonate the hydroxyl groups of the anhydrous glucose units. On the other hand, in anhydrous medium such as *N*,*N*-dimethyl formamide (DMF), CD easily reacts with sodium hydride (NaH) resulting in deprotonation at the hydroxyl group mainly at the C2-position of the AGU (Rong & D'Souza, 1990). Alternatively by intramolecular nucleophilic substitution with the oxygen atom at the 3-position, the CD oxoanion converts to 2,3-cyclodextrin epoxide (Khan & D'Souza, 1996).

In this study, cyclodextrin polymers were prepared by cross-linking β-cyclodextrin with maleic anhydride through the oxoanion (or epoxide) intermediate prepared by NaH. Different reaction conditions such as temperature, time and molar ratio of the reagents were investigated in terms of

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Table 1
Weight-average molecular weight and percent ratio of the β-CD polymerization products prepared at different reaction temperatures and times (molar ratio of β-CD:NaH:maleic anhydride was 1:7:7, and percent ratio of the peaks was calculated based on RI responses for each peaks; serially connected size exclusion columns (TSK Gel 3000 PW, 4000 PW, and 5000 PW) were used for analysis) (% ratio)

	4 h	12 h	24 h
25°C	1300 (13%) 6500 (67%)	ND[a]	1300(47.5%) 2300 (40.7%)
60°C	2700 (15%) 9100 (80%) 43,000 (2.4%)	ND	1900 (12%) 3800 (50%) 17,000 (20%) 46,000 (5.3%)
100°C	7200 (17.5%) 12,000 (43%) 18,000 (17%) 88,000 (19%)	12,000 (8.4%) 31,000 (15%) 47,000 (24%) 88,000 (27 %) 288,000 (14%)	17,000 (5.3%) 28,000 (11%) 78,000 (49%) 380,000 (18%)
130°C	225,000 (17.2%) 466,000 (40.6%)	250,000 (14.6%) 530,000 (39%)	ND

[a] Not determined.

molecular size, chemical structure and water solubility of the polymers.

2. Materials and methods

2.1. Materials

Crystalline β-CD was donated by DAESANG Co., Ltd. (Seoul, South Korea). Maleic anhydride (MA), *N*,*N*-dimethyl formamide (DMF) and Linde type 4A (molecular sieve) were purchased from Showa Chemicals (Tokyo, Japan). DMF was predried under Linde type 4A, and then distilled under vacuum. The dried DMF was stored in a dark bottle with Linde type 4A (Armarego & Perrin, 1996). Sodium hydride was purchased from Aldrich Chemicals (Milwaukee, WI, USA).

2.2. Polymerization

β-CD (1.135 g, 0.001 mol) was dissolved in DMF (25 ml), and then solid NaH (0.001–0.007 mol) was slowly added into the solution with vigorous stirring. Stirring was continued for 24 h at room temperature. The solution became clear, but yellow-green colored and gumlike when the CD and NaH molar ratio was 1:4 or higher. This gumlike product could be manno-2,3-epoxy-β-cyclodextrin (Khan & D'Souza, 1996). Into the β-CD oxoanion or epoxide solution, solid MA (0.001–0.011 mol) was slowly added. The reaction mixture was continuously stirred in a sealed round bottom flask in an oil bath at a controlled temperature (25–130°C) up to 24 h. The reaction product was precipitated and washed with a large quantity of acetone, and finally dried in a vacuum desiccator at room temperature to a white to yellow-brown powder.

2.3. Water solubility

Water solubility of the CD polymers prepared at a molar ratio of 1:7:7 for CD:NaH:MA but at different temperatures (25, 60, 100 and 130°C) for the reaction of CD and MA was compared. Each sample (0.2 g) was dispersed in water (0.8 ml) for 1 h at room temperature, and then the mixture was centrifuged at 10,000 rpm for 15 min. The supernatant was taken and dried into anhydrous solids using a speed vacuum drier (Heto-Holten, Allerod, Denmark), and the dry mass was measured for the calculation of solubility.

2.4. ^{1}H-NMR spectroscopy

^{1}H-NMR spectra of the CD polymers were obtained using a Bruker NMR spectrometer (500 MHz, USA Bruker Instruments, Mountain View, CA). For analysis, the CD polymer samples were dissolved in D_2O containing 0.75% 3-(trimethylsilyl)propionic-2,2,3,3-d_4 acid sodium salt as an internal reference (0 ppm).

2.5. Molecular weight analysis

A HPSEC system equipped with a multiangle laser-light scattering and refractive index detectors was used to determine the weight-average molecular weights (M_w) of the CD polymers. The instruments and analytical procedure were the same as reported by You, Fiedorowicz and Lim (1999). Three size exclusion columns (TSK Gel 3000PW, 4000PW, and 5000PW, 7.8 × 600 mm, Tosoh, Tokyo, Japan) were used for the separation based on the hydrodynamic size. The weight-average molecular weight (M_w) of the chromatographic fractions was calculated by the Debye method using Astra 4.50 software (Wyatt Technology, Santa Barbara, CA). The d*n*/d*c* value (change in RI with concentration) of the CD polymers was assumed identical to the value for unreacted β-CD (0.137).

3. Results and discussion

3.1. Water solubility

All polymer samples prepared at a temperature below 130°C and at a molar ratio of 1:7:7 for CD:NaH:MA were completely dissolved in water at 1:4 sample:water ratio. This result indicates that the water solubility of the products was higher than 20%. However, the dry mass analysis after centrifugation revealed that the CD polymers prepared at 130°C for 4 and 12 h were relatively insoluble (7.2 and 4.2%, respectively). The soluble fractions would be the small oligomers or unpolymerized CD derivatives, and the majority of the polymers were comprised in the insoluble fractions. The difference in solubility between the two reaction temperatures may indicate that the polymerization reaction of CD with MA occurred in a greater yield at 130°C.

Table 2
Weight-average molecular weight and percent ratio of the β-CD polymerization products prepared at different molar ratio of β-CD:NaH:MA (reaction time and temperature were 12 h and 80°C, respectively; TSK gel 3000PW column was used for analysis) (% ratio)

β-CD:MA	β-CD:NaH			
	1:1	1:2	1:4	1:7
1:1	1300 (23%) 1700 (10.3%) 1970 (10.3%) 3560 (6.1%)	1250 (11.6%) 2160 (3.7%) 3880 (18%)	1200 (18.3%) 2100 (13.1%) 2300 (10.5%)	1260 (20.3%) 1780 (7.9%) 1830 (8.4%) 6550 (1.1%)
1:2	1400 (27.7%) 1600 (19.4%) 2770 (12.8%) 5330 (10.4%)	1300 (38.8%) 1500 (18.2%) 2800 (19.6%)	1650 (22.8%) 2100 (12.9%) 4200 (8.5%)	1300 (21.7%) 1600 (16.6%) 2000 (20.3%) 5500 (7.1%)
1:4	1400 (20.6%) 1500 (27.5%) 2300 (14.7%) 5100 (24.8%)	1500 (24.6%) 1700 (15.4%) 2700 (10.7%) 6330 (35.3%)	1600 (14%) 1800 (14.6%) 2100 (14.5%) 7270 (21.3%)	1350 (12.1%) 1500 (24.6%) 2700 (29.2%) 6800 (17.8%)
1:7	1800 (31%) 2600 (16.7%) 3900 (19.3%) 13,500 (17.4%)	2300 (24.9%) 3500 (10.4%) 5900 (28.7%) 19,950 (17.8%)	1800 (25.7%) 3450 (6.1%) 6200 (34.4%) 27,300 (15.5%)	2500 (7.9%) 3500 (8.7%) 5400 (63.4%) 60,000 (15.8%)
1:11	1800 (26.9%) 2600 (15.7%) 3400 (25.1%) 12,600 (22.4%)	4000 (17.2%) 9400 (54.9%) 42,000 (22.5%)	3800 (19.3%) 18,700 (43.8%) 104,000 (21.7%)	4100 (8.4%) 20,000 (55.5%) 170,000 (31.6%)

3.2. Molecular weight

The weight-average molecular weight and percent ratio of each chromatographic fraction of the polymerization products under different reaction conditions are presented in Tables 1 and 2.

Although large quantities of NaH and MA (seven times the mass of CD) were used for the reaction, polymerization appeared inefficient when the MA was reacted at 25 or 60°C (Table 1). Only a slight amount of the polymers with M_w greater than 40,000 Da was detected when the reaction temperature was 60°C (2.4 and 5.3% at 4 and 24 h, respectively). However, the polymer fractions with high M_w dramatically increased as the reaction temperature was raised to 100°C. At this temperature, CD monomer was no longer found in the reaction mixture.

Fig. 1 shows how the chromatogram of the CD polymer products changed as the reaction time was increased at

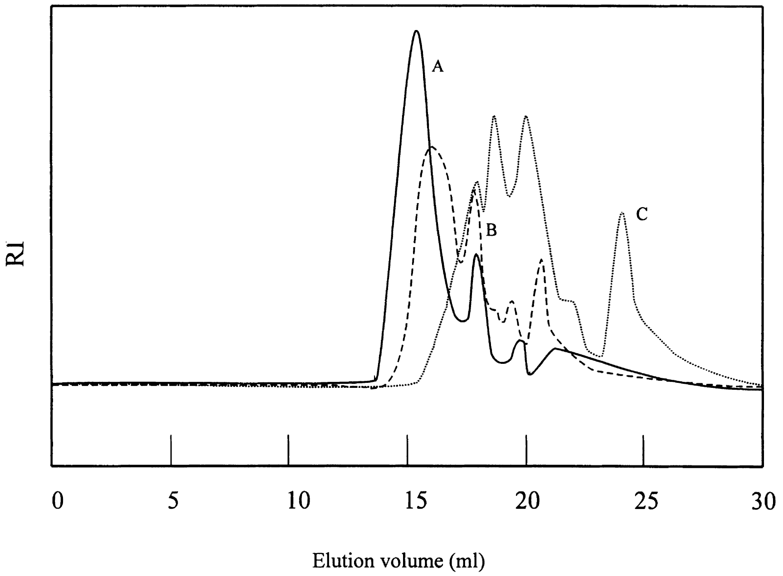

Fig. 1. HPSEC chromatograms of β-CD polymerization products prepared at different reaction times (A: 24 h, B: 12 h, and C: 4 h). Molar ratio of β-CD:NaH:MA was 1:7:7, and reaction temperature was 100°C.

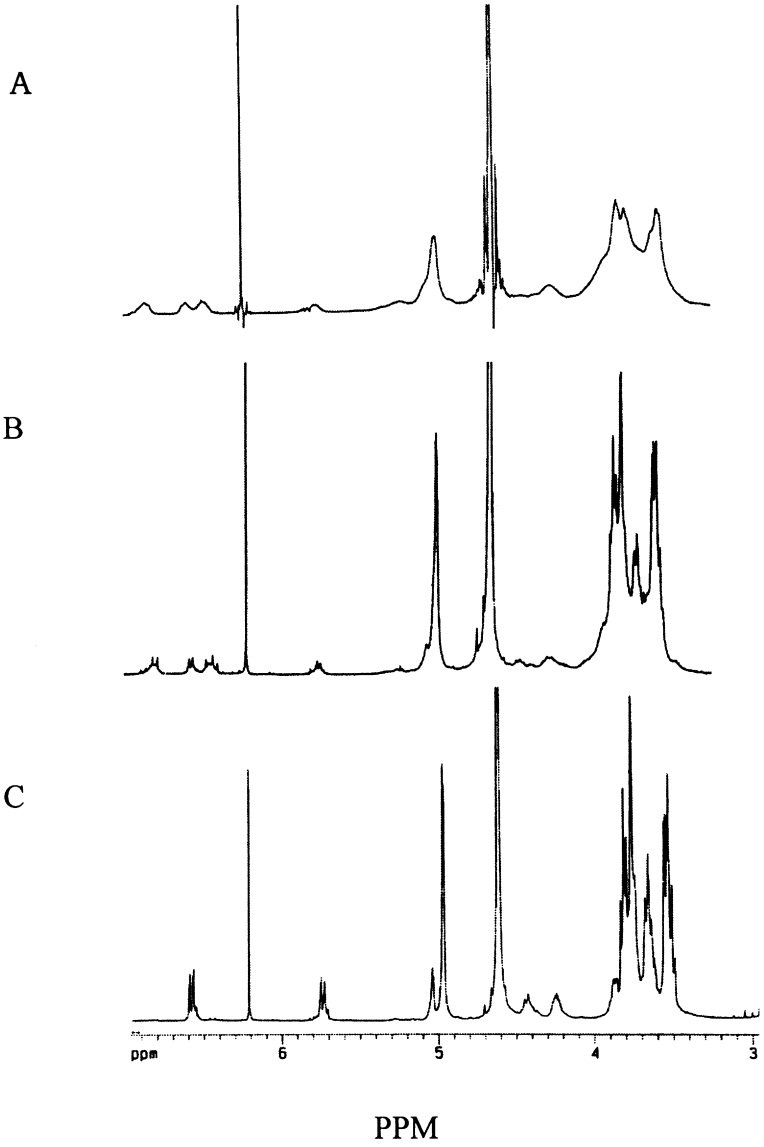

Fig. 2. ^{1}H-NMR spectra of β-CD polymers prepared at different reaction times (A: 24 h, B: 12 h, and C: 4 h). Molar ratio of β-CD:NaH:MA was 1:7:7, and reaction temperature was 100°C.

100°C. Each reaction product consisted of several peaks with distinctively different M_w values under a high performance size-exclusion chromatography. The reaction occurred in heterogeneous or discontinuous patterns. Based on the chromatogram, CD dimers (approximate M_w 2000 Da) or small oligomers were synthesized at the early stage of the reaction, and these products interacted to display a higher M_w range from 20,000 to 90,000 Da as the reaction proceeded. After 12 h of the reaction, a major peak appeared in an elution range of 14–17 ml (B in Fig. 1). This peak was shifted to a much higher M_w region with increased intensity when the reaction continued to 24 h (C in Fig. 1). These major peaks of high M_w in the 12 and 24 h products could be divided into two fractions based on the light scattering chromatograms (curves not shown). The M_w values for the two fractions were 78,000–88,000 and 288,000–380,000 Da as shown in Table 1.

At 130°C, the M_w values for polymer products were much greater than the values measured with the products prepared at 100°C (Table 1). Even by the 4 h reaction, the smallest fraction had the M_w value greater than 200,000 Da. With an extended reaction period (12 h), however, the chromatographic pattern (data not shown) and the calculated M_w values were not significantly changed (Table 1). Therefore, the polymerization reaction was almost completed within 4 h at 130°C under the given reaction conditions. More than half of the reaction products had M_w over 200,000 Da. This result, with the substantial increase in M_w as the reaction temperature was raised from 100 to 130°C, agreed with the insolubilization by the reaction at 130°C as previously indicated.

The effect of molar ratio between β-CD, NaH and MA on the M_w profile of the CD polymers was also examined at a fixed reaction temperature (80°C) and time (12 h). Table 2 shows that the increase in NaH addition leads to the increase of M_w. This trend appeared more significant when CD and maleic anhydride (MA) ratio was 1:7 or 1:11. Therefore, the precedent oxoanion formation by NaH was very important in facilitating the CD cross-linking reactions with MA. When the CD:MA molar ratio was 1:11, the NaH effect appeared more significant than when it was 1:7 (Table 2). At the ratio of 1:11, butenedioic β-CD dimers (M_w 2600 Da) were detected when the CD:NaH ratio was 1:2. At the highest ratio of 1:7:11 for CD:NaH:MA, the majority (87%) of the products had the M_w value of 20,000 or greater.

Based on the M_w distribution of the β-CD polymers obtained under the wide range of reaction conditions used, it can be concluded that NaH produces mono and multi-oxoanions or epoxides in a β-CD molecule, depending on the amount of NaH added. The multi-oxoanionic β-CD derivatives could react with more than two maleic anhydrides, and form polymers with butenedioic ester bridges. Therefore, the molar ratio of the reagents was also important in determining the structure and molecular weight of the CD polymer.

3.3. ^{1}H-NMR spectroscopy

^{1}H-NMR spectra are consistent with the proposed reaction for butenedioic ester formation. The CD polymer products prepared at 100°C for MA reaction contained mixtures of β-CD mono- and poly-butenedioic esters based on the NMR spectra (Fig. 2). The vinylic proton signals at 6.6 and 5.7 ppm which showed almost equivalent integrated peak areas were assigned as the α and β protons, respectively, adjacent to ester linkage of monoestered free acids. The singlet peak at 6.2 ppm could be assigned to be two identical vinylic protons in the butenedioic diester moieties between CD molecules. As the reaction temperature increased, the vinylic proton for diester linkages (δ 6.2) was intensified whereas the two proton signals for monoesters (δ 5.7 and 6.6) became minor (Fig. 2). This result indicates that the cross-linking reaction of the β-CD oxoanions with maleic anhydride become prevalent as the reaction period increased. It could be suggested that the free acids of butenedioic monoesters, which were produced initially, were gradually consumed for the following cross-linking reactions with CD oxoanions. The molecular weight increase with the reaction time caused the reduced resolution for NMR spectrum as shown in Fig. 2.

The NMR spectra for the reactions for 12 or 24 h (A or B) showed two signals which were not observed with the sample at 4 h (C) appeared approximately at 6.4 and 6.9 ppm. It was assumed that these signals represented also the vinyl protons of maleic monoesters. The chemical shift difference might be originated from the esterification on the different hydroxyl groups of AGU in the CD.

Two signals were found around 5 ppm and these represented the two anomeric protons of the AGU in β-CD moiety on the spectra. The small singlet at δ 5.05 could be assigned as oxoanion or manno-epoxide of the AGU in β-CD (Khan & D'Souza, 1996), and the other major doublet signal at δ 4.97 with coupling constants $J_{1-2} = 3.2$ Hz represented the anomeric protons in intact AGU. More experiments will be needed for detailed characterization of these CD polymers.

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References

Armarego, W. L., & Perrin, D. D. (1996). *Purification of laboratory chemicals*, (pp. 192–193). Oxford: Butterworth-Heinemann.

Bender, M. L., & Komiyama, M. (1978). In K. Hafner & J.-M. Lehn & C. W. Rees & P. R. Schleyer & B. M. Trost & R. Zahradnik (Eds.), *Cyclodextrin chemistry*, (pp. 2–9). New York: Springer.

Cserhati, T., & Forgacs, E. (1994). Retention of some monoamine oxidase inhibitory drugs on a β-cyclodextrin polymer-coated silica column. *Journal of Chromatography*, *660*, 313–318.

Cserhati, T., Fenyvesi, E., & Szejti, J. (1992). Interaction of nonylphenyl and tributylphenyl ethylene oxide ionic surfactants with highly soluble cyclodextrin derivatives. *Journal of Inclusion Phenomenon*, *14*, 181–188.

Harada, A., Furue, M., & Nozakura, S. (1975). Preparation of cyclodextrin-containing polymers and their catalysis in ester-hydrolysis. *Journal of Polymer Science, Polymer Letters Edition*, *13*, 357–360.

Harada, A., Furue, M., & Nozakura, S. (1976). Cyclodextrin-containing polymers. 1. Preparation of polymers. *Macromolecules*, *9*, 701–704.

Hinze, W. L. (1981). *Separation and Purification Methods*, *10*, 159–237.

Khan, A. R., & D'Souza, V. T. (1996). Epoxides of the secondary side of cyclodextrins. *Journal of Organic Chemistry*, *61*, 8301–8303.

Rong, D., & D'Souza, V. T. (1990). A convenient method for functionalization of the 2-position of cyclodextrins. *Tetrahedron Letters*, *31*(30), 4275–4278.

Shaw, P. E., & Wilson, C. W. (1985). Reduction of bitterness in grapefruit juice with β-cyclodextrin polymer in a continuous-flow process. *Journal of Food Science*, *50*, 1205–1207.

Shaw, P. E., Tatum, J. H., & Wilson, C. W. (1984). Improved flavor of novel orange and grapefruit juices by removal of bitter components with β-cyclodextrin polymer. *Journal of Agriculture and Food Chemistry*, *32*, 832–836.

Su, C. S., & Yang, C. P. (1991). Partial removal of various food components from aqueous solution using crosslinked polymers of cyclodextrin with epichlorhydrin. *Journal of Science and Food Chemistry*, *54*, 635–643.

Szejtli, J. (1982). *Cyclodextrin and their inclusion complexes*, (pp. 95–140). Budapest, Hungary: Akadémiai Kiadó.

Szejtli, J. (1988). *Cyclodextrin technology*, (pp. 48–58). Dordrecht, The Netherlands: Kluwer Academic.

You, S., Fiedorowicz, M., & Lim, S.-T. (1999). Molecular characterization of wheat amylopectins by multiangle laser light scattering analysis. *Cereal Chemistry*, *76*(1), 116–121.