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Synthesis of chitosan derivatives bearing cyclodextrin and adsorption of *p*-nonylphenol and bisphenol A

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Abstract

An insoluble crosslinked chitosan bearing cyclodextrin moieties was prepared by a one-step procedure with *N*-succinylated chitosan and *mono*-6-amino-mono-6-deoxy-β-cyclodextrin in the presence of the water-soluble carbodiimide. The degree of substitution by the CD moiety achieved 0.27 with the addition of DMF to the reaction solution. The obtained material adsorbed bisphenol A quicker than *p*-nonylphenol.

Keywords: Chitosan; Cyclodextrin; Crosslinking; Bisphenol A; *p*-Nonylphenol

1. Introduction

Recently, it was pointed out that some chemicals have the potential to disrupt endocrines. The removal of these compounds from waste water or polluted soil is considered to be important for environmental protection, because some of these chemicals, especially, *p*-nonylphenol, bisphenol A and dialkyl phthalate are abundantly used in industry as materials for nonionic surfactants, component of polymers, and plasticizers for plastics, respectively.

Due to the hydrophobic character of these molecules, many kinds of pollutants are expected to be included in the cavities of cyclodextrins (CDs), cyclomalto-oligosaccharides which are known as inclusion compounds forming agents consisting of 6, 7, and 8 α-glucopyranose units, by the hydrophobic interaction. Therefore, some researchers reported the synthesis of the polymers having the CD moiety (CD-P) and showed they have the potential to remove toxic compounds from water (Szejtli, 1988).

On the other hand, the preparations of CD-P were reported based on the interest in the synthesis of artificial receptors (Asanuma, Akiyama, Kajiya, Hishiya, & Komiyama, 2001) or the removal of undesired compounds from food (Asanuma, Shibata, Kakazu, Hishiya, & Komiyama, 1997). Chitosan has been used for the syntheses of functional polymers having pendant CD moieties (Auzely-Velty & Rinaudo, 2001; Furusaki, Ueno, Sakairi, Nishi, & Tokura, 1996; Kurauchi, Ono, Wang, Egashira, & Ohga, 1997; Tanida et al., 1998; Tojima et al., 1998). However, these materials were soluble in water and it was necessary to crosslink the chitosan before connecting the CD for the application as an adsorbent in water (Ishigami et al., 1995; Nishiki, Tojima, Nishi, & Sakairi, 2000; Sreenivasan, 1998; Tojima et al., 1999). In studies obtaining insoluble CD–chitosan by preceding crosslinking, the CD contents were relatively low. The main reason for the limited CD content can be assumed to that the reactions for binding CD to chitosan were carried out in heterogeneous systems.

In this paper, we describe the synthesis of CD-P using *N*-succinyl chitosan (Suc-Chitosan). Suc-Chitosan becomes a self-crosslinked gel by the treatment with a condensation reagent (Hirano & Moriyasu, 1981). We succeeded in the immobilization of CD moiety onto the chitosan molecules and these products were insoluble in water without any further crosslinking treatment. One of the advantages of our method is that reaction begins under homogeneous conditions allowing samples with a high CD content to be obtained.

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2. Experimental

2.1. Preparation of ACD

Mono-6-amino-*mono*-6-deoxy-β-cyclodextrin (ACD) was synthesized via the mono-6-tosylated β-cyclodextrin (TsCD) according to the method reported by Takahashi, Hattori, and Toda (1984). The yield was 22% from the native β-CD. Elemental analysis; calcd for $C_{42}H_{67}O_{34}N \cdot 6H_2O$, C, 40.61%, H, 6.74%, N, 1.13%; *found*, C, 40.67%, H, 6.79%, N, 1.11%. FAB-MS, $m/z = 1134$ ($[M+1]^+$).

2.2. Preparation of N-succinyl chitosan

Chitosan (purchased from Nakarai Tesque, degree of deacetylation was 0.73 by elemental analysis and weight average molecular weight was 860,000 by GPC) was succinylated by the method reported by Hirano and Moriyasu (1981). Four gram of chitosan was dissolved in a 5% aqueous acetic acid solution (80 ml) and then the solution was diluted with 320 ml of methanol. A prescribed amount of succinic anhydride, dissolved in a minimum amount of acetone, was added to the chitosan solution. The reaction solution was vigorously stirred for 16 h at room temperature. The obtained viscous solution was diluted with water (400 ml) and the pH of the solution was adjusted to 10 using 2 M NaOH solution. After dialysis for 5 days, the solution was concentrated by a rotary evaporator, and freeze-dried to obtain a cotton-like white material.

2.3. Preparation of CD-P

In a typical procedure, the CD-grafted chitosan (CD-P) was synthesized as follows: 0.085 g (0.40 mmol) of the succinylated chitosan and 0.567 g (0.50 mmol) of the ACD were stirred in 3 ml water for 1 h. One ml of a 10% solution of 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) 0.52 mmol was then added to the reaction solution. The reaction solution was stirred for 48 h. Hydrochloric acid was added to the reaction solution to adjust the pH to 2 and the solution was dialyzed in a dialysis tube against distilled water for 5 days. The content of the dialysis tube was freeze-dried to yield 0.159 g of a cotton-like white material. The contents of the CD moiety in CD-P were determined by the phenol–sulfuric acid method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956). This Colorimetric method is specific for neutral sugars and not sensitive to amino-sugars like glucosamine units in chitosan. Degree of substitution by the CD moiety (DS_{CD}) of the above product was 0.16 (CD content, 45 wt%).

2.4. Adsorption experiments

Fifty ml of an aqueous solution of 1.23×10^{-5} M *p*-nonylphenol (NP) or 4.8×10^{-4} M bisphenol A (BPA), and 50 mg of CD-P or activated carbon (granules water treatment grade) for comparison, were put into an Erlenmeyer flask. After the flask was shaken at 90 rpm at 25 °C for a prescribed time, 3 ml of the solution was removed and the concentrations of NP and BPA were determined by absorbance at 208 and 276 nm, respectively. The solution was returned to the flask after the measurement. The kinetic parameters were calculated from the time course of the concentrations of NP and BPA using a simple model.

3. Results and discussion

3.1. Characterization of Suc-Chitosan

It is logical to use a hydrophilic rather than hydrophobic polymer skeleton to remove hydrophobic chemicals from water by binding to CD substituents. It is a hydrophobic polymer was used hydrophobic chemicals would bind to the polymer rather than the CD. Suc-Chitosan is such a hydrophilic polymer with reactive amino and carboxyl groups. These functional groups are available not only for inter- or intramolecule crosslinkings to easily yield a hydrogel under the appropriate conditions (Hirano & Moriyasu, 1981), but also for connecting the CD moieties.

Suc-Chitosan was prepared according to the method reported by Hirano and Moriyasu (1981). The degree of succinylation, defined as the average number of succinyl groups per repeating units of chitosan, was calculated from the elemental analysis data. The degrees of succinylation of the samples are shown in Table 1. The degree of succinylation achieved 0.59 with an excess amount of succinic anhydride in Run A. Hirano et al. reported that the degree of succinylation was an important factor for the formation of the gel; Suc-Chitosan with too many or few succinyl groups could not produce the insoluble hydro-gel (Hirano & Moriyasu, 1981). Therefore, Suc-Chitosan obtained in Run B was used in the following experiments.

Table 1
Succinylation of chitosan with succinic anhydride

Run	Chitosan (g)	Succinic anhydride (g)	Time (h)	Yield (g)	Degree of succinylation
A	4.00	26.41	9.5	6.36 (159%)	0.59
B	4.00	4.00	9.0	4.82 (120%)	0.43

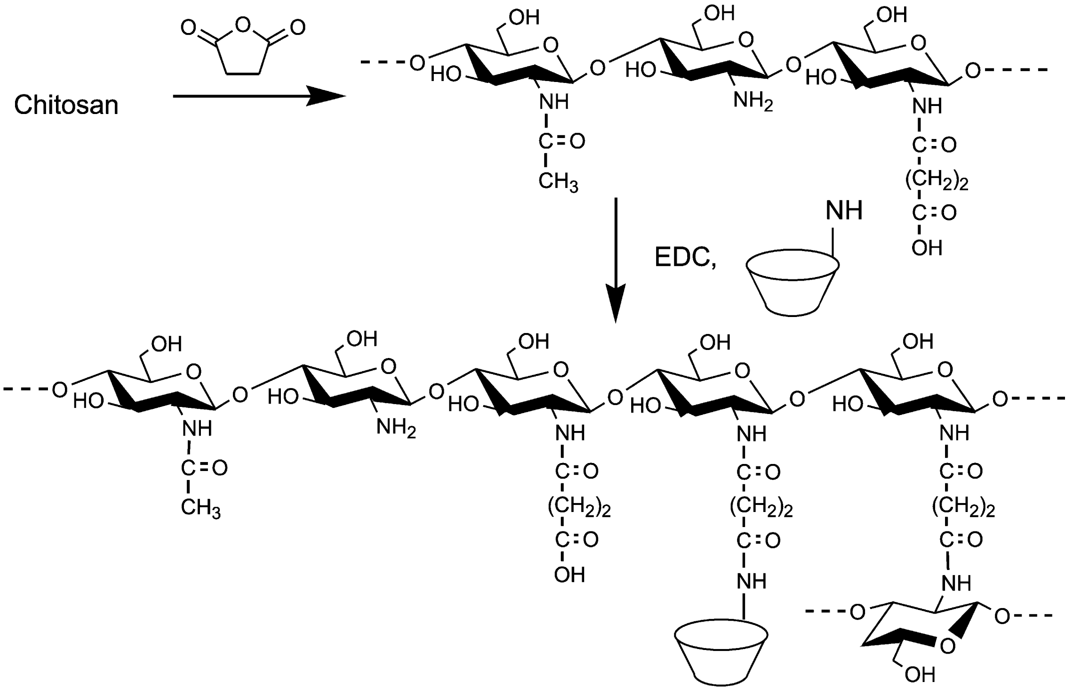

Fig. 1. Preparation of Chitosan bearing β-CD (CD-P) from *N*-succinyl chitosan (Suc-Chitosan).

3.2. Synthesis of CD-P

The coupling reaction of ACD and Suc-Chitosan was carried out using EDC, a kind of water-soluble carbodiimide (Fig. 1). $\mathrm{DS}_{\mathrm{CD}}$, the average number of CD moiety per repeating unit of chitosan, of the CD-Ps obtained under various conditions are plotted in Fig. 2. With a small amount of EDC, the $\mathrm{DS}_{\mathrm{CD}}$ of the products was under 0.05. The amounts of EDC and ACD were increased to obtain products with a higher $\mathrm{DS}_{\mathrm{CD}}$. With the EDC concentration of 0.13 M, the $\mathrm{DS}_{\mathrm{CD}}$ of the CD-P ranged from 0.15 to 0.19. An increase in the amount of ACD was not very effective because $\mathrm{DS}_{\mathrm{CD}}$ increased only slightly as the amount of ACD used increased. The reason is unclear, but it probably comes from the solubility of ACD; when the molar ratio of ACD to the repeating units of Suc-Chitosan was 5, the reaction solution was turbid. The addition of 20–50 vol% DMF was examined to improve the solubility of ACD. While Suc-Chitosan was precipitated when the amount of added DMF was greater than 35 vol%, the $\mathrm{DS}_{\mathrm{CD}}$ became 0.28 with 50 vol% DMF. Even if the amount of DMF was only 20 vol%, the $\mathrm{DS}_{\mathrm{CD}}$ of the product was 0.20, higher than those under the corresponding condition without DMF.

The FT–IR spectrum of CD-P was compared with those of ACD and Suc-Chitosan (Fig. 3). The spectrum of CD-P showed peaks ascribable to both ACD (O–H, 3400 cm^{-1}, C–O–C, 1030 cm^{-1}) and the Suc-Chitosan skeleton (N–H, 2400–3500 cm^{-1}, C=O, 1650 cm^{-1} and N–H, 1560 cm^{-1}).

Our strategy is convenient because the insoluble CD-P was obtained in a one-step reaction. Similar materials consisting of CD and chitosan have been previously reported (Kurauchi et al., 1997; Tanida et al., 1998). These materials had a higher $\mathrm{DS}_{\mathrm{CD}}$ than that in this paper, 0.82 (Kurauchi et al., 1997) and 0.59 (Tanida et al., 1998), respectively, but the products were soluble in water. In order to obtain an insoluble material, crosslinking of the chitosan was carried out with a crosslinker before introducing the CD moiety to the chitosan (Ishigami et al., 1995; Nishiki et al., 2000; Tojima et al., 1999). In the heterogeneous reaction systems, the CD contents were limited to approximately 34 wt% (β-CD, Ishigami et al., 1995), 0.16 of $\mathrm{DS}_{\mathrm{CD}}$ (β-CD, Tojima et al., 1999), and 0.085 of $\mathrm{DS}_{\mathrm{CD}}$ (β-CD, Nishiki et al., 2000). It was considered that CD-P in this study was crosslinked between the carboxyl groups and amino groups on the Suc-Chitosan skeleton. Kurauchi et al. used *N*-carboxymethylated chitosan (Kurauchi et al., 1997) similar to Suc-Chitosan. In their case, however, crosslinking

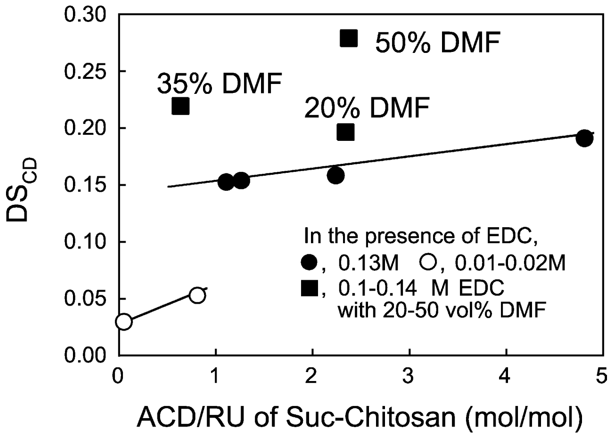

Fig. 2. Effect of reaction condition on $\mathrm{DS}_{\mathrm{CD}}$.

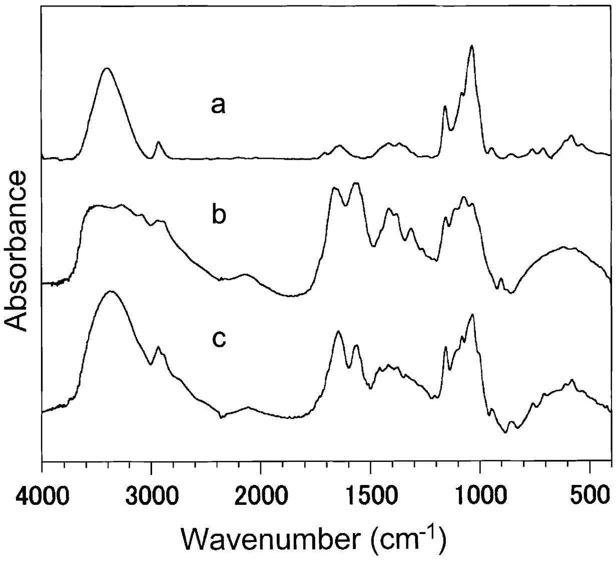

Fig. 3. FT–IR spectra of (a) ACD, (b) Suc-Chitosan, and (c) CD-P.

did not occur because most of the amino groups were used for the *N*-carboxymethylation.

3.3. Adsorption behavior of CD-P

Results of the adsorption experiments using BPA and NP are plotted in Fig. 4(a) and (b), respectively. The initial concentration of BPA and NP in Fig. 4 were different because the solubility of NP is quite low. Fig. 4(a) shows that CD-P adsorbed 80% of the BPA in the solution while only a small amount of BPA was adsorbed to Suc-Chitosan crosslinked without CD moiety. This indicated that the adsorption mainly occurred with the CD moiety and that the adsorption onto the hydrophilic chitosan skeleton was negligible. The amount of adsorbed BPA onto CD-P at equilibrium was consistent with that expected from the amount of the CD moiety in CD-P used in the experiment (molar ratio, CD/BPA = 0.85:1). In the case of the dilute NP solution (1.23×10^{-5} M), CD-P adsorbed most of the NP because CD was in excess to the NP (molar ratio, CD/NP = 33:1). Fig. 4(a) and (b) show that the initial adsorption rates of the CD-P with BPA and NP were larger than that of activated carbon (AC).

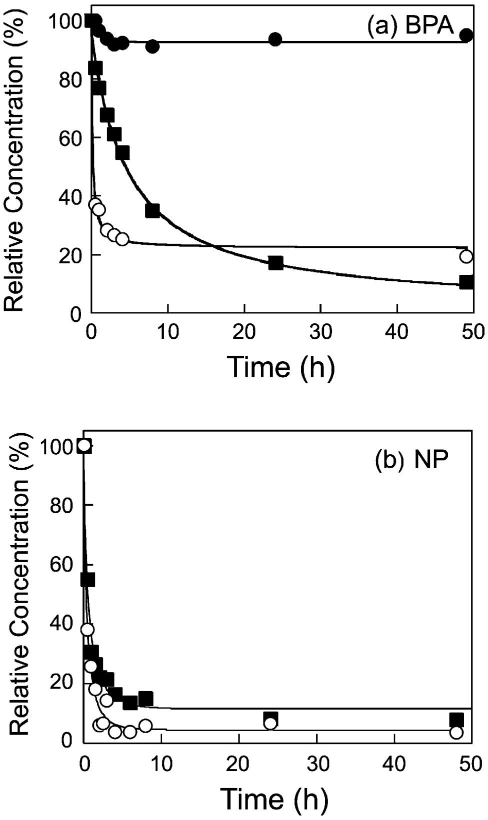

Fig. 4. Adsorption of (a) 4.8×10^{-4} M BPA and (b) 1.23×10^{-5} M NP. ●: Suc-Chitosan (4.8×10^{-5} M), ○: CD-P, ■: AC.

Table 2 shows the kinetic parameters, k_1, and k_{-1}, the concentration of substance at equilibrium, y_e, and the equilibrium constant, K, calculated by an approximation using an assumed simple adsorption model (Eq. (1)).

$$[\mathrm{A}] + [\mathrm{S}] \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} [\mathrm{A{\cdot}S}] \tag{1}$$

where [A] is the apparent concentration of the adsorption site, [S] is the concentration of the adsorption substance, and [A·S] is the apparent concentration of the adsorption site which adsorbed substances. This model is expected to agree with the behavior of the CD-P as well, because a CD moiety is considered to be able to include one molecular substance. On the other hand, the adsorption behavior of AC cannot be completely described with this model because of the various sizes of the adsorption sites.

The k_1 for the adsorption of BPA onto CD-P was 8.6 times larger than that of NP onto CD-P while the k_1 for the

Table 2
Kinetic parameters of adsorption of BPA and NP onto CD-P and AC

	BPA				NP			
	K_1 ($\mathrm{M^{-1}s^{-1}}$)	k_{-1} ($\mathrm{s^{-1}}$)	y_e/y_0 (%)	K ($\mathrm{M^{-1}}$)	k_1 ($\mathrm{M^{-1}s^{-1}}$)	k_{-1} ($\mathrm{s^{-1}}$)	y_e/y_0 (%)	K ($\mathrm{M^{-1}}$)
CD-P	6.6×10^{-1}	6.4×10^{-6}	22	1.0×10^{5}	7.7×10^{-2}	1.4×10^{-6}	1.2	5.6×10^{4}
AC	1.7×10^{-2}	1.9×10^{-4}	1.7	8.7×10^{1}	6.0×10^{-2}	5.4×10^{-3}	0.43	1.1×10^{1}

For the values in the case of AC, the saturated adsorption amount of phenol was tentatively used as the initial apparent concentration of the adsorption site.

adsorption of BPA onto AC was 0.28 times that for NP onto AC. It was concluded that CD-P has some selectivity for the adsorption of BPA due to the CD moiety. This finding agreed with the result reported by Tanida et al. (1998).

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