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Original Article

Improved Synthesis of Chitosan-Bearing β-Cyclodextrin and Its Adsorption Behavior towards Bisphenol A and 4-Nonylphenol

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Abstract

The synthesis of chitosan-bearing β-cyclodextrin (CDC) has been improved by use of an alternative condensing agent, 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMT-MM). CDC was obtained by the reaction of succinyl chitosan with a small amount of *mono*-6-amino-*mono*-6-deoxy-β-cyclodextrin (ACD) within a shorter reaction time. Under optimal conditions, the CD yields reached to >50%, which were almost the same as those in the reactions using 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC). The gelation time suggests that the crosslinking of chitosan and the immobilization of the ACD occur competitively. To test the potential of CDC for selective removal of pollutants, the adsorption of bisphenol A (BPA) and 4-nonylphenol (NP) from aqueous solution onto CDC was investigated. In the case of the equilibrium concentrations more than 10 mg/L, the amounts of both sorbents onto CDC were close to the estimated saturated value of the CDC (85 mg/g for BPA and 81 mg/g for NP).

Introduction

Recently, it has been reported that some kinds of substances produced abundantly in industry, such as 4-nonylphenol (NP), disrupt endocrines in various wild animals and humans [1]. It is suspected that the effect of these substances in dilute solutions is not negligible, so that many researchers are developing new selective adsorbents for effective removal of the pollutants from water or soil.

α-, β-, and γ-cyclodextrins (CDs), comprising 6, 7, or 8 α-glucopyranose units, respectively, are well-known hosts that can include various organic compounds. Since some organic pollutants are hydrophobic and fit well with the CD cavity [2], some researchers have reported the application of CDs for the removal of pollutants including endocrine disrupting compounds [3, 4]. In order to use CDs for removing pollutants in aqueous media, it is important to make them insoluble in water. The immobilization of CDs onto insoluble supports has been regarded as an appropriate way to obtain insoluble CD materials. We previously reported the synthesis of an insoluble chitosan derivative bearing CD moieties (CDC) using 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) as a condensing agent. The resulting CDC adsorbed both bisphenol A (BPA) and NP well [5]. Chitosan was used because it is a reactive polymer and its hydrophilicity is advantageous to avoid unselective adsorption of hydrophobic compounds onto chitosan through hydrophobic interaction.

On the other hand, some researchers have used other CDCs as chiral separators [6], controlled-releasing agent of bioactive substances [7], and adsorbent of endocrine disruptors [4]. Since the uncrosslinked CDCs [4, 6, 7] tend to dissolve in water, crosslinking of the chitosan, usually before immobilization of CDs, was often reported. The merit of our method [5] is that the immobilization of CD moieties and the crosslinking of the chitosan are carried out simultaneously and the insoluble material containing 50 wt% of β-CD is obtained without further crosslinking treatment. However, it required a long reaction time and relatively large amounts of CD derivative the condensing agent, EDC.

In this study, we describe the improved synthesis of CDC using 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMT-MM) instead of EDC. DMT-MM, developed by Kunishima *et al.* [8] can be used as a superior water-soluble condensing agent for the formation of amide linkages from carboxyl and amino groups with satisfactory yields in aqueous systems. CDC was obtained by the reaction with

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DMT-MM and a smaller amount of CD within a much shorter reaction time as compared with the case of EDC. We also describe the adsorption behavior of CDC with BPA and NP which follows the Langmuir equation.

Experimental

Materials

β-CD, purchased from Wako Pure Chemicals Industries, Ltd., was dried at 100 °C under reduced pressure for 12 h and then used for preparation of *mono*-6-amino-*mono*-6-deoxy-β-cyclodextrin (ACD). Chitosan (Nakarai tesque Inc., MW_n 562,000, MW_w 860,000, degree of deacetylation, 0.73) was used for the synthesis of succinylated chitosan. 2-Chloro-2,6-dimethoxy-1,3,5-triazin (Tokyo Kasei Co., Ltd.), bisphenol A(BPA), and 4-nonylphenol (NP) were used as received.

Analysis

Elemental analysis was carried out by a 2400 CHN Elemental Analyzer (Perkin Elmer Inc.). The concentrations of BPA and NP in adsorption experiments were determined by UV–Visible absorption (U-3000 spectrophotometer; Hitachi, Ltd.) and fluorescence (F-4010 fluorescence spectrophotometer; Hitachi, Ltd.) spectroscopy, respectively.

CD contents of the products were estimated by the phenol-sulfuric acid method [9], which is sensitive to neutral sugars, such as anhydroglucose units in CDs, but not to amino-sugars such as the anhydroglucosamine units in chitosan. Details of the procedure were described in our previous paper [5]. The degree of substitution of the CD moieties (DS_{CD}), which corresponds to an average number of CD moieties on a repeating unit (RU) of succinyl chitosan, was calculated from the CD content.

Synthesis

4-(4,6-Dimethoxy-1,3 5-triazin-2-yl)-4-methylmorpholinium chloride (DMT-MM)

The DMT-MM was prepared from 2-chloro-2,6-dimethoxy-1,3,5-triazin and *N*-methylmorpholine by the method reported by Kunishima *et al.* [8]. DMT-MM was isolated as a white powder by filtration, washed with tetrahydrofuran, and used for the further reactions after drying. DMT-MM was identified by FAB-MS and NMR.

Mono-6-amino-mono-6-deoxy-β-CD (ACD)

ACD was synthesized according to the method reported by Takahashi *et al.* [10].

Succinyl Chitosan (SucC)

Chitosan was succinylated with succinic anhydride [11]. The degree of succinylation of the succinyl chitosan (D_{SUC}), the average number of *N*-succinyl groups per anhydroglucosamine unit of chitosan, was calculated from the C and N contents derived from elemental analysis. Samples of SucC with D_{SUC} of 0.49 and 0.53 were used for the synthesis of chitosan-bearing β-CD.

Chitosan-bearing β-CD (CDC)

CDC was synthesized according to the method [5] previously reported by using DMT-MM instead of EDC (Scheme 1). In a typical procedure, SucC (0.1 g) was dissolved in distilled water (14 mL). ACD (0.25 g) was added to the solution and the mixture was stirred until the solution became clear. Aqueous DMT-MM solution (1 mL) was added to the solution and stirred at room temperature. As the reaction proceeded, the reaction mixture became a clear gel. After the prescribed time, the gel was placed in a dialysis tube and dialyzed against distilled water for 5 days. The content of the tube was freeze-dried to give a sponge-like CDC, which was washed with ethanol and finally dried under reduced pressure.

Adsorption experiments

A prescribed amount of CDC (DS_{CD}, 0.14) and 10 mL of BPA or NP aqueous solution were placed in a glass tube (20 mL) and shaken (90 rpm) at 25 °C for 24 h. The mixture in the glass tube was filtered off and the concentrations of BPA and NP in the filtrate were determined from absorbance (275 nm) and fluorescence intensity (304 nm; excitation 221 nm), respectively. The amounts of BPA and NP adsorbed on CDC were calculated by the concentration of the filtrate. Details of the experimental conditions are summarized in Table 3.

Results and discussion

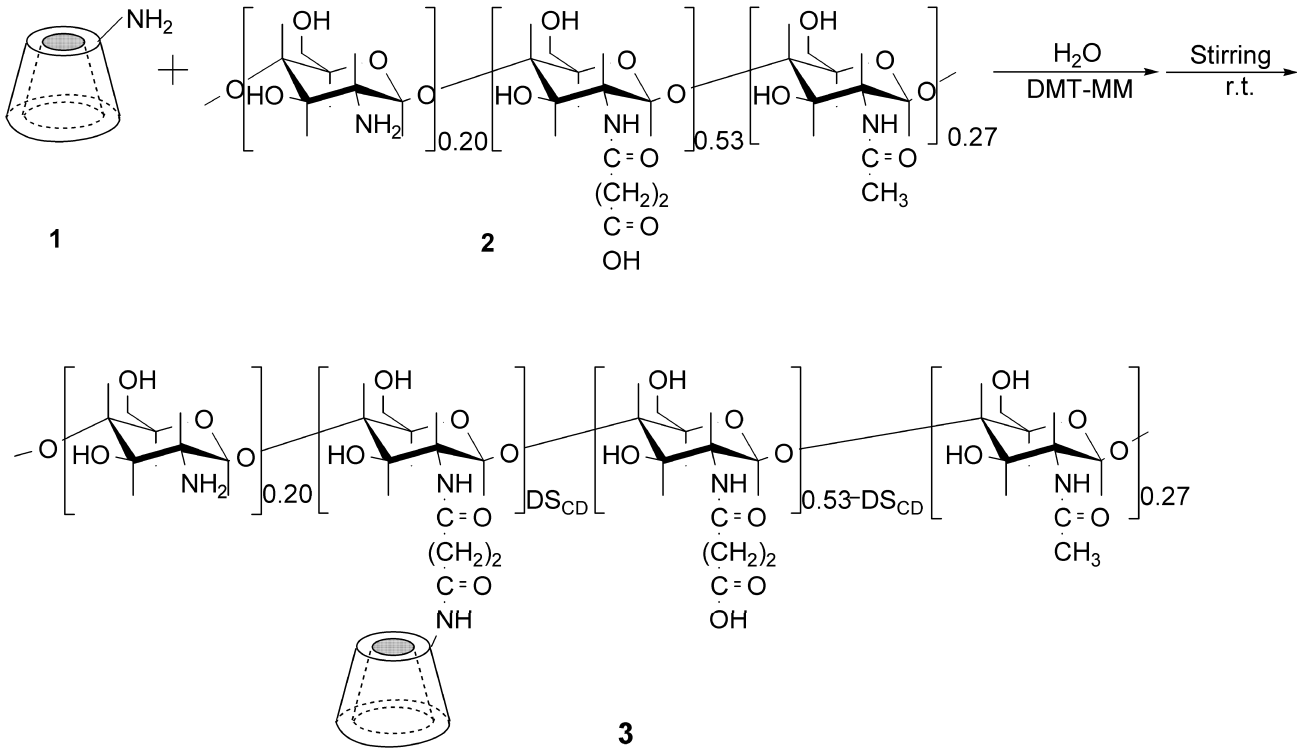
Synthesis of CDC

Time course of DS_{CD} of CDC

In a preliminary experiment, an aqueous solution of SucC turned to a hydro-gel within 10 min after addition of DMT-MM. This is attributed to the intermolecular crosslinking between the amino and carboxyl groups on the SucC molecules as in the case of EDC [5]. Considering that the degree of acetylation of the original chitosan is 0.27, the degrees of substitution of the remaining free amino groups in SucC are 0.24 and 0.20, respectively (Table 1). The free amino group is considered essential to obtain the crosslinked, insoluble material employed in this work.

Figure 1 shows the time courses of CD contents of CDC.

In this paper, we discuss the CD content of the CDC using DS_{CD}, which corresponds to an average number of CD moieties on a RU of succinyl chitosan. The DS_{CD} values of the CDC obtained in the system in the



Scheme 1.

Table 1. Yields and elemental analyses of succinylated chitosan

	Elemental analyses				
Yield (g)	C%	H%	N%	Dsuc	Conversion (%)
5.03 (125%)	36.82	6.73	5.05	0.49	67
4.32 (99.6%)	38.67	6.54	5.20	0.53	73

presence of an excess amount of DMT-MM ([DMT-MM]/[COOH on SucC] = 1.21) are higher than those in the presence of a smaller amount of DMT-MM ([DMT-MM]/[COOH on SucC] = 0.82). The DS_{CD} leveled off 2 h after the beginning of reaction, even when using the differing amounts of DMT-MM described.

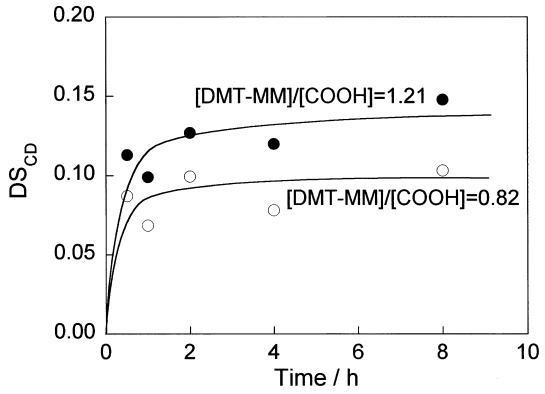


Figure 1. Time course of DS_{CD} with various amounts of DMT-MM. The values of the DS_{SUC} of SucC were 0.53 (●) and 0.49 (○) and the reactions were carried out under the conditions described in Table 2 (●, run 1 and ○, run 2).

Several researchers reported uncrosslinked CDC with higher DS_{CD}, such as, 0.82 [6] and 0.59 [12]. These materials, however, were soluble in water so that it was impossible to use them as adsorbents in aqueous media. The insoluble CDC samples were also prepared by crosslinking of chitosan before immobilization of CDs. However, the CD contents of the insoluble CDC reported were relatively low, 34 wt% of the whole material [13], $DS_{CD} = 0.16$ [14], and $DS_{CD} = 0.085$ [4], perhaps owing to the immobilization of CD under heterogeneous conditions. Our method is advantageous to obtain the insoluble CDC with a high CD content, because the reaction was treated in a homogeneous system.

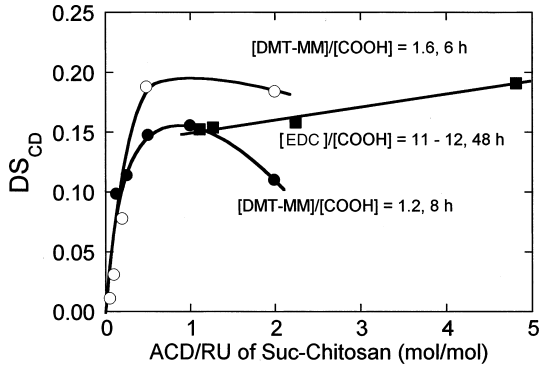


Figure 2. DS_{CD} of CDC obtained with various amounts of ACD. The D_{SUC} values of SucC were 0.49 (●), 0.53 (○), and 0.43 (■) [5] and the reactions using DMT-MM were carried out under the conditions described in Table 2 (●, runs 3–7 and ○, runs 8–12). The amounts of EDC was 0.54 mmol (■, [EDC]/[COOH on SucC] = 11). The reactions were carried out for 8 h (●), 6 h (○), and 48 h (■), respectively.

Table 2. Amounts of the reagents in the syntheses of CDC

Run	SucC			ACD		DMT-MM		
	D_{Suc}	(g)	COOH (mmol)	(g)	(mmol)	(g)	(mmol)	[DMT-MM]/[COOH in SucC]
1	0.53	0.10	0.23	0.25	0.22	0.08	0.28	1.21
2	0.49	0.10	0.22	0.25	0.22	0.050	0.18	0.82
3	0.53	0.10	0.24	0.063	0.055	0.078	0.28	1.20
4	0.53	0.10	0.24	0.125	0.11	0.078	0.28	1.20
5	0.53	0.10	0.24	0.250	0.22	0.078	0.28	1.19
6	0.53	0.10	0.24	0.501	0.44	0.078	0.28	1.20
7	0.53	0.05	0.12	0.500	0.44	0.039	0.14	1.20
8	0.49	0.10	0.22	0.025	0.022	0.10	0.36	1.62
9	0.49	0.10	0.22	0.050	0.044	0.10	0.36	1.62
10	0.49	0.10	0.22	0.101	0.088	0.10	0.36	1.63
11	0.49	0.10	0.23	0.250	0.22	0.10	0.36	1.60
12	0.53	0.05	0.12	0.501	0.44	0.05	0.36	1.53
13	0.53	0.10	0.23	0.25	0.22	0.03	0.12	0.49
14	0.53	0.10	0.23	0.25	0.22	0.08	0.28	1.20
15	0.53	0.10	0.24	0.25	0.22	0.12	0.45	1.91
16	0.53	0.10	0.24	0.25	0.22	0.20	0.71	3.01
17	0.53	0.10	0.24	0.25	0.22	0.53	0.27	4.07

In our previous study using EDC [5], a large amount of ACD was required to obtain CDC with a high CD content. The DS_{CD} of the product obtained using DMT-MM was investigated using various amounts of ACD. In Figure 2, the values of DS_{CD} are plotted against molar ratios of RU of succinyl chitosan and ACD in the reaction systems for comparison with the results in the case of EDC (■) [5]. The reactions were carried out for 6 h (○) and 8 h (●) with DMT-MM and for 48 h with EDC (■); the molar ratios of the condensing agent to carboxyl group in SucC were 1.63 (○), 1.20 (●) and 11 (■). Even if the differences in reaction conditions were considered, we can conclude that the CDC having a DS_{CD} comparable to that of EDC can be obtained using smaller amount of DMT-MM and a shorter reaction time as compared with the case of EDC (Table 2).

Effect of the amount of ACD on gelation time

It was observed that the gelation occurred much faster with DMT-MM in the presence of ACD than with EDC. Figure 3 shows the effect of [ACD]/[SucC] on the gelation time. In these experiments, the gelation time is defined as the time required for the formation of a self-supporting gel. The presence of such a gel was confirmed visually by inverting the reaction apparatus. Figure 3 shows that DS_{CD} becomes large as the increase of the amount of the ACD added and reaches to 0.19 (CD

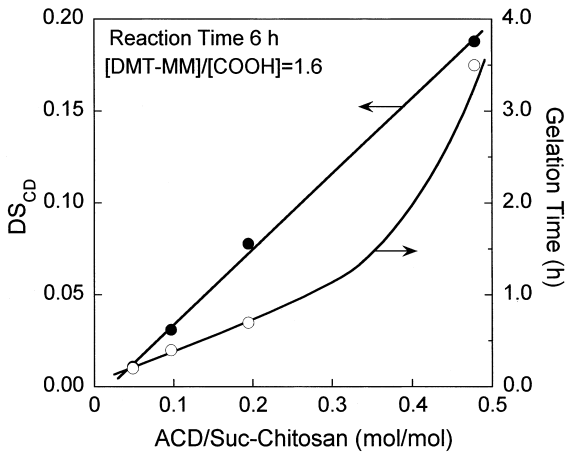


Figure 3. Effect of amount of ACD on gelation time (○). The reaction conditions are shown as runs 8–11 in Table 2. DS_{CD} of the samples obtained after 6 h (●) are also plotted.

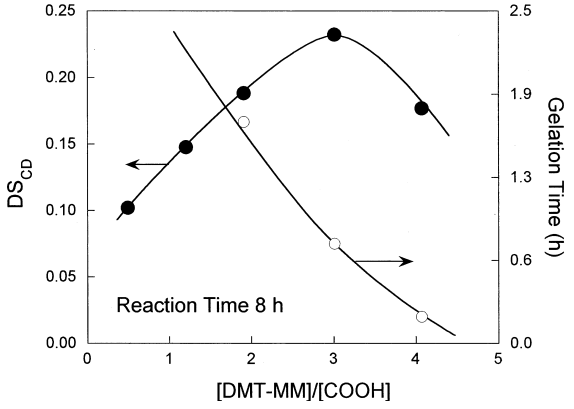


Figure 4. Effect of amount of DMT-MM on CD content (●) and gelation time (○). SucC (0.1 g, DS_{SUC} 0.53) and ACD 0.25 g ([ACD]/[repeating unit of SucC] = 1.1) were stirred in the presence of DMT-MM for 8 h. The reaction conditions are shown as runs 13–17 in Table 2. The gelation obtained in runs 13 and 14 were not observed within 8 h.

content, 49 wt%) at [ACD]/[repeating unit in SucC] of 0.48. Furthermore, the gelation time became longer as the amount of added ACD was increased. This seems to imply that the crosslinking of SucC and the reaction of amino groups of ACD occur competitively.

Effect of the amount of DMT-MM on CD content and gelation time

Figure 4 shows the effect of the amount of DMT-MM on the CD content of the CDC and the gelation time of the reaction solution. As the amount of the DMT-MM was increased, the CD content rose until the molar ratio of DMT-MM to the carboxyl group in SucC became ca. 3. When the molar ratio of DMT-MM to the carboxyl group in SucC was 4, however, the CD content was smaller than that in the case of the molar ratio of 3. Gelation of the reaction solution was not observed within 8 h when the molar ratio of DMT-MM was <2. Gelation occurred within 15 min when the molar ratio of DMT-MM was 4. The reason for the decrease in DS_{CD} value at the molar ratio of DMT-MM = 4 is obscure, but it might be caused by slow heterogeneous reaction occurring after gelation.

Adsorption of BPA and NP onto CDC

Figure 5 shows the adsorption isotherm of the CDC observed in batch experiments using BPA and NP as adsorbates. In the case of the equilibrium concentrations

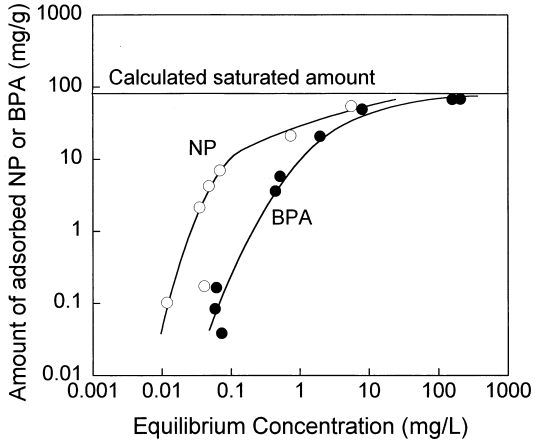


Figure 5. Adsorption isotherms of BPA and NP on CDC. Experimental conditions are summarized in Table 3.

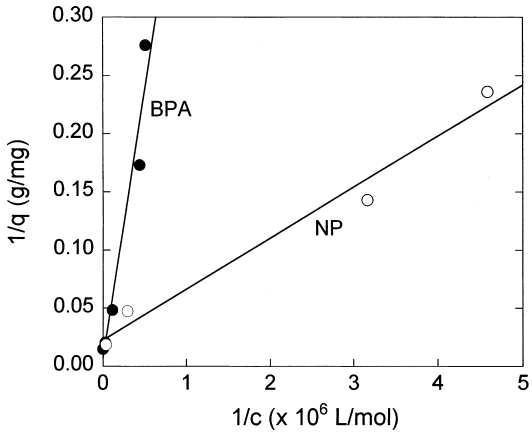


Figure 6. Langmuir adsorption isotherms of CDC.

Table 3. Results of the adsorption experiments with various concentrations of BPA and NP

Adsorbates (mol/L)	CDC (mg)	Initial molar ratio (CD/adsorbate)	Equilibrium conc. (mol/L)	Amounts of adsorption (μmol/g)	CD used in sorption (mol%)	Removal efficiency[a] (%)
BPA 1.00×10^{-6}	40.03	1490	3.22×10^{-7}	0.182	0.20	67.8
	20.03	745	2.59×10^{-7}	0.370	0.10	74.1
	10.02	373	2.71×10^{-7}	0.678	0.05	72.9
1.00×10^{-5}	100.05[b]	37.4	(0.00[c])	(2.28[c])	(2.67[c])	(100[c])
	5.07	18.9	1.95×10^{-6}	1.59	4.27	80.5
	3.06	11.4	2.26×10^{-6}	25.3	2.07	77.4
1.00×10^{-4}	10.04	3.74	8.73×10^{-6}	64.5	57.84	91.3
	3.01	1.12	3.52×10^{-5}	302	24.43	64.8
1.00×10^{-3}	10.03	0.37	7.01×10^{-4}	90.4	80.32	29.9
	3.04	0.11	9.09×10^{-4}	984	80.16	9.08
NP 1.00×10^{-6}	40.12	1493	(0.00[c])	(0.06[c])	(0.07[c])	(100[c])
	20.20	752	5.38×10^{-8}	0.463	0.12	94.6
	10.20	383	1.86×10^{-7}	0.787	0.21	81.2
1.00×10^{-5}	10.17	37.8	1.59×10^{-7}	9.64	2.60	98.4
	5.08	18.9	2.19×10^{-7}	19.2	5.18	97.8
	3.05	11.4	3.15×10^{-7}	31.7	8.54	96.9
1.00×10^{-4}	10.08	3.75	3.36×10^{-6}	95.4	25.77	96.6
	3.02	1.12	2.55×10^{-5}	246	66.32	74.5

[a] Removal Efficiency (%) = [adsorbate after adsorption]/[adsorbate before adsorption] × 100.
[b] 100 mL of solution was used.
[c] Calculated by assuming that all BPA or NP was adsorbed.

more than 10 mg/L, the adsorption amounts of both sorbents onto CDC were close to the estimated saturated value of the CDC (85 mg/g for BPA and 81 mg/g for NP). When the concentration of adsorbates was lower (1 mg/L of BPA or 0.1 mg/L of NP), the amounts of adsorption were relatively small (about 0.1 mg/g).

Details of the results of the adsorption experiments are summarized in Table 3. Judging from these results, the efficiency of adsorption of NP is a slightly higher than that of BPA. In the case of adsorption of NP from 10^{-4} mol/L solution (22 ppm), CDC adsorbed 95.4 mmol/g while silica-supported CD adsorbent [3] adsorbed 1.7–6.4 mmol/g from a 15 ppm solution. We also found that the molar ratio of CD used in adsorption to CD in CDC sample was 25.8 mol% in the case of CDC, which is much larger than the corresponding values of silica-supported CD [3], 3.4–4.1 mol%. These findings are explained by the higher availability of CD moieties bound to highly hydrophilic chitosan skeleton compared with the silica-supported CD [3].

Figure 5 indicates that the adsorption isotherms are of the typical Langmuir type, as described by the following equation,

$$1/q = 1/q_e + 1/(q_e \times K \times c),$$

where q, q_e, c and K are the amounts of the observed adsorbed adsorbates (mg/g), the amount of the adsorbates adsorbed at equilibrium, the equilibrium concentration (mol/L), and the equilibrium constants $(\text{mol/L})^{-1}$, respectively. Some of the data in Table 3 are used for the Langmuir plot of Figure 6, which suggests that the adsorption behavior of CDC obeys the above equation as the Langmuir type. In other words, one molecule of the adsorbate is trapped by one CD moiety. This is in agreement with the fact that, as shown in Figure 5, the adsorbed amounts at higher equilibrium concentration are close to the calculated saturation amount of the adsorption. Furthermore, we conclude that adsorption onto chitosan chain is negligible. These findings suggest that the selectivity of CDC in adsorption depends on its CD moiety. The adsorption equilibrium constants calculated from Figure 6 are summarized in Table 4. The obtained equilibrium constant, K, for NP was 32 times larger than K for BPA.

Figure 7 shows the plots of the amounts of adsorbed BPA and NP in the case of dilute solution using linear scale. A linear relation between the amount of adsorption and the equilibrium concentration was observed. This agrees with the adsorption behavior oftenly observed in dilute solution experiments, which frequently follows Henry's equation,

$$q_e = K_P \times c,$$

Table 4. Adsorption equilibrium constants of CD chitosan

	BPA	NP
q_e (mg/g)	1.39×10^2	4.50×10
K $(\text{mol/L})^{-1}$	1.58×10^4	5.07×10^5

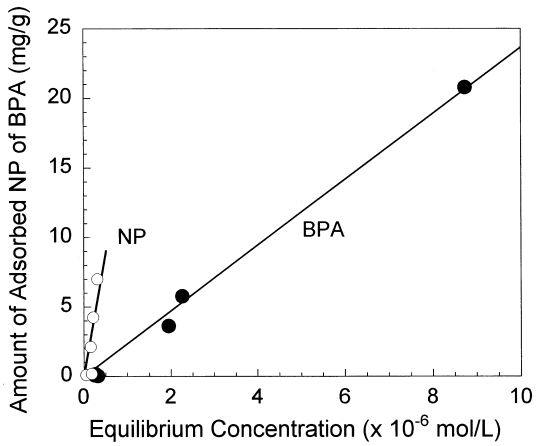


Figure 7. Adsorption isotherms in a low equilibrium concentration region.

where q_e, c and K_p are the amounts of the adsorbed adsorbates at equilibrium (mg/g), the equilibrium concentration (mol/L), and equilibrium constant $(\text{mol/L})^{-1}$, respectively.

References

1. H. Yokota, M. Seki, M. Maeda, Y. Oshima, H. Tadokoro, T. Honjo, and K. Kobayashi: *Environ. Toxicol. Chem.* **20**, 11, 2552 (2001).
2. N. Kawasaki, M. Araki, T. Nakamura, and S. Tanida: *J. Colloid Interf. Sci.* **238**, 215 (2001).
3. T.N.T. Phan, M. Bacquet, and M. Morcellet: *React. Funct. Polym.* **52**, 117 (2002).
4. M. Nishiki, T. Tojima, N. Nishi, and N. Sakairi: *Carbohydr. Lett.* **4**(1), 61 (2000).
5. N. Aoki, M. Nishikawa, and K. Hattori: *Carbohydr. Polym.* **52**, 219 (2003).
6. Y. Kurauchi, H. Ono, B. Wang, N. Egashira, and K. Ohga: *Anal. Sci.* **13** (February), 47 (1997).
7. R. Auzely-Velty and M. Rinaudo: *Macromol.* **34**(11), 3574 (2001).
8. M. Kunishima, C. Kawachi, J. Morita, K. Terao, F. Iwasaki, and S. Tani: *Tetrahedron* **55**, 13159 (1999).
9. M. Dubois, K.A. Gilles, J.K. Hamilton, P.A. Rebers, and F. Smith: *Anal. Chem.* **28**(3), 350 (1956).
10. K. Takahashi, K. Hattori, and F. Toda: *Tetrahedron Lett.* **25**(31), 3331 (1984).
11. S. Hirano and T. Moriyasu: *Carbohydr. Res.* **92**, 323 (1991).
12. F. Tanida, T. Tojima, S.-M. Han, N. Nishi, S. Tokura, N. Sakairi, H. Seino, and K. Hamada: *Polymer* **39**(21), 5261 (1998).
13. H. Ishigami, T. Okemoto, K. Arai, S. Yoshida, K. Sato, and I. Yamamoto: Japanese Kokai Tokkyo Koho, JP07173201, 6 pp. (1995).
14. T. Tojima, H. Katsura, M. Nishi, S. Tokura, and N. Sakairi: *Carbohydr. Polym.* **40**, 17 (1999).