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Preparation of Insoluble Chitosan Beads Functionalized by Carboxymethylated β-Cyclodextrin

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Insoluble spherical chitosan beads bearing β-cyclodextrin moieties (CDC beads) were prepared by a condensation between multiply carboxymethylated β-cyclodextrin and chitosan beads without any further crosslinking. The shape of the original chitosan beads was retained when the reaction was carried out at pH 9 or pH 10 but the reaction at pH 7 and pH 8 gave powder and highly swelled product, respectively. The CDC beads adsorbed more than 85% of bisphenol A from 10^{-5} mol/L aqueous solution while the chitosan adsorbed only 2 %. The CDC beads also adsorbed 4-nonylphenol and 4-nonylphenol ethoxylates from aqueous solution. We concluded that the CD moieties worked both as crosslinker and as adsorption sites. We also confirmed that almost all of the bisphenol A and 4-nonylphenol adsorbed was released from the CDC beads by a treatment with 50% aqueous ethanol solution.

Key words: adsorbent, carboxymethyl β-cyclodextrin, chitosan, bisphenol A, 4-nonylphenol

1. INTRODUCTION

Cyclodextrins, cyclic oligosaccharides consisting of 6 – 8 glucose residues, are known as inclusion forming compounds. The CDs have been used mainly in food industry. Recently, polymers with CD moieties have been applied to environmental use, especially adsorption of the hydrophobic compounds from water [1-5]. From an interest for selective adsorbent for endocrine disrupting chemicals, such as, bisphenol A (BPA) and 4-nonylphenol (4-NP), we reported a derivative of chitosan bearing β-CD moieties (CDC)[4 and 5].

Previous to our study, several researchers have reported synthesis, association and adsorption behavior of CDC [3, 6-9]. According to those studies, it was revealed that chitosan derivative bearing CD moieties as pendant groups tends to be soluble in water [6-8]. In order to obtain insoluble CDC available as an adsorbent in water, several researchers carried out crosslinking treatment before binding CD to the chitosan. CD content of the insoluble CDC, however, have been relatively low, for example, 0.085 [3] and 0.1[9] of degree of substitution. The low CD content is probably due to that the reactions of the crosslinked insoluble chitosan were carried out under heterogeneous condition and/or that the amino groups available for the immobilization of the CD moieties was consumed by the crosslinking.

In our previous study [4 and 5], we applied the self-crosslinking of partially *N*-succinylated chitosan to obtain the insoluble chitosan bearing β-cyclodextrin. The reaction was initiated under homogeneous condition so that the crosslinking and the grafting CD moieties occurred simultaneously to give the insoluble CDC with CD content more than 50 wt%. We also found that the CDC could adsorb hydrophobic compounds such as BPA and 4-NP that chitosan itself does not adsorb.

Our method was easy and the CD content of the CDC was satisfactory high but the product was a fragile cotton-like material. Considering use as a column packing, fine beads are more attractive.

Here we describe a preparation of insoluble CDC beads using condensation between multiply carboxmethylated β-CD (CM-CD) and chitosan beads. In this strategy, the CM-CD works not only as adsorption site for hydrophobic compounds but also as a crosslinking agent to give the insoluble product in a spherical bead shape. We also investigated adsorption behavior of the CDC beads using BPA, 4-NP, and 4-nonylphenol ethoxylates.

2. EXPERIMENT

2.1 Materials

Chitosan beads (2.7 mm in diameter, water content 97wt%) were prepared from acidic solution of chitosan (degree of deacetylation, 0.73) according to the method written in a literature [10]. The CM-CD was obtained by a reaction between β-CD and monochloroacetic acid [11]. Anaverage number of carboxymethyl groups on a CM-CD molecule was approximately 3.4 and the CM-CD included unmodified β-CD and carboxymethylated β-CDs bearing 1-6 carboxylmethyl groups. The CM-CD was used for the condensation with chitosan beads without further purification. 4-(4,6-Dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMT-MM) was synthesized by the method described by Kunishima *et al.*[12]

2.2 Synthesis

A typical procedure for preparation of the CDC beads was follows: a prescribed amount of chitosan beads was stirred in a buffer solution. DMT-MM and CM-CD were added as an aqueous solution in this order and then the mixture was stirred. After the reaction, the product was isolated by filtration and washed with distilled water. CD content of the product was determined after freeze-drying. The CDC beads had been stored in water until adsorption experiments.

2.3 Analysis

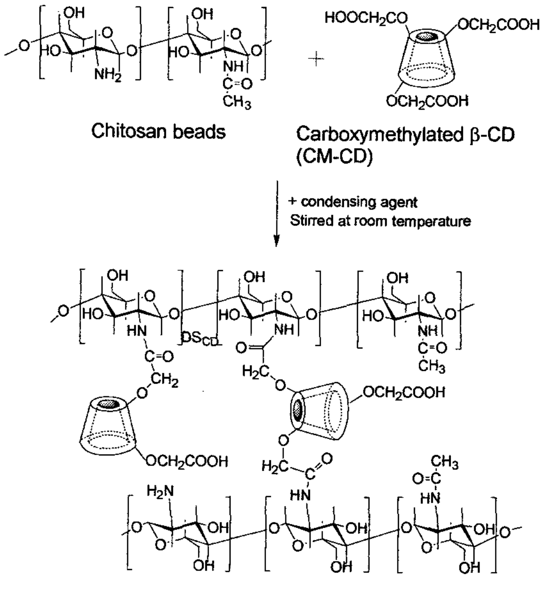
The CD content of the obtained CDC was determined by phenol-sulfuric acid method, a specific colorimetric method for neutral sugars [13]. The concentrations of BPA, 4-NP, and 4-nonylphenol ethoxylates (NPEs) in their solution were determined by UV-Visible absorption (U-3000 spectrophotometer; Hitachi, Ltd.)

3. RESULTS AND DISCUSSION

3.1 Preparation of CDC beads

Our strategy for preparation of the insoluble CDC beads was summarized in Scheme 1. One of carboxymethy group on CM-CD molecules probably reacted with amino groups on chitosan beads to give pendant CD moieties at first. Then the other carboxymethyl groups on the CM-CD reacted to the remaining amino groups on the chitosan beads to result in crosslinking. In order to avoid the dissolution of the chitosan beads bearing pendant CD moieties in early stage[6-8], the reaction condition should be the one where the condensations proceed quickly to pass the non-crosslinked state.

The CD contents of the products obtained by the reaction between chitosan beads and CM-CD were plotted in Figure 1 against molar ratio of DMT-MM to number of repeating units (RU) in the chitosan beads. The values of the CD contents were larger than 10% in all experiments, satisfactory high in comparison with the previous studies using crosslinked chitosan beads [3 and 9]. However, appearance of the products strongly depended on pH of the reaction solution. The products obtained at pH 7 were powder, not beads, because the original beads dissolved completely and then deposited later as the reaction proceeded. During the reaction at pH 8, the chitosan beads did not dissolve but became highly swelled. Therefore, the products were larger than the original beads, not spherical, and were very fragile. On the other hand, when the pH of the reaction solution was kept over pH 9, the dissolution of CD-grafted chitosan did not occur and the products were obtained with the original spherical bead shape. The CD content of the product depended on an amount of DMT-MM at pH 9 and pH 10 and reached about 28%. The CD contents of the products obtained at pH 7 and 8 were higher than at pH 9 and 10 when amount of DMT-MM was small. It could be explained by that the reaction proceeded smoothly under homogeneous (pH 7) and highly swelled (pH 8) state. Furthermore, the dissolution-deposition phenomenon at pH7 supports that the expected crosslinking by the CM-CD moieties as showed in Scheme 1 occurred via chitosan derivative bearing pendant CD moieties.



Scheme 1 Preparation of insoluble CDC beads using multiply carboxymethylated β-CD

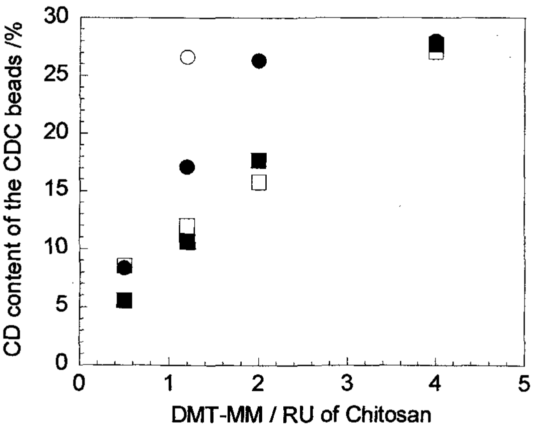


Figure 1 CD contents of the CDC beads obtained using DMT-MM at pH 7 (●), pH 8 (○), pH 9 (■), and pH 10 (□).

The CD contents of the products obtained at pH 9 were plotted in Figure 2 against molar ratio of the CM-CD to RU in chitosan. When the molar ratio of CM-CD was larger than one, the CD contents leveled off at about 27% which was the almost same as the maximum value in Figure 1. The reason for the limited CD content is not certain but it might be caused by limited amount of accessible amino groups under the heterogeneous reaction conditions.

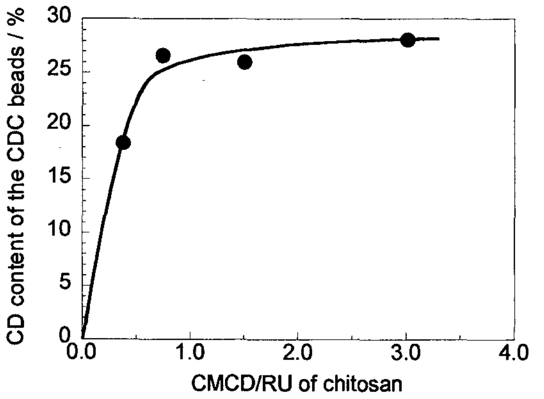


Figure 2 CD contents of the CDC beads obtained using various amount of CM-CD.

Figure 3 shows a time course of the CD content of the CDC beads in pH 10. The leveling off in CD content was also observed at about 16% after 4 hours from the beginning of the reaction. In our previous study [5], it took about 4 hours to obtain an insoluble hydrogel of CDC from partially *N*-succinylated chitosan. The required time to obtain the CDC beads in this study was not so longer than that for the CDC in our previous study [5].

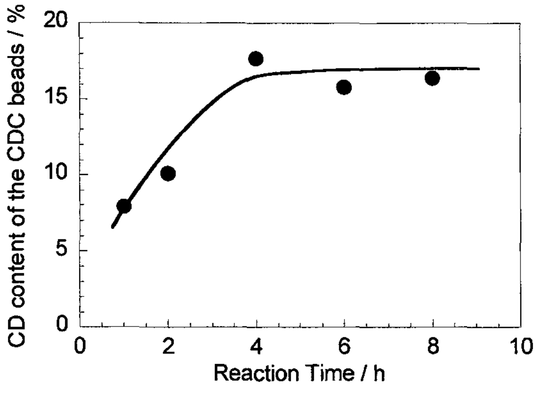


Figure 3 Time course of the CD contents of the CDC beads obtained at pH 10.

3.2 Adsorption of BPA and NP

Concentration of BPA after the adsorption for 24 hours using 30 mg of carious CDC beads and 30 mL of BPA aqueous solution (10^{-5} mol/L) was plotted in Figure 4 as adsorption ratio, ratio of amount of adosorbed BPA against that in the solution before adsorption. As the cotton-like CDC in our previous study [5], all kinds of the CDC beads adsorbed more than 85% of BPA in the solution while unmodified chitosan beads adsorbed only 2% of BPA. This means that the adsorption was occurred on CD moieties, that is, the CD moieties worked not only as crosslinker but also as adsorption sites. Because the amount of CD moieties in the used CDC beads were much excess for BPA in the solution even if CD content was only 8.5%, the differences in adsorption ratio according to the CD contents were small under this adsorption condition.

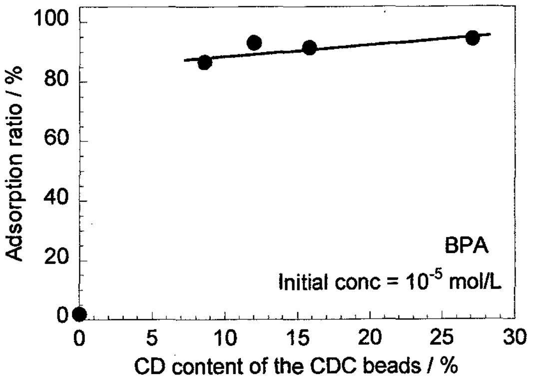


Figure 4 Adsorption of BPA onto CDC beads with various CD contents.

Adsorption isotherm of the CDC beads was investigated using BPA and 4-NP under various conditions. Results were plotted in Figure 5 as a Freundlich plot. Amount of adsorption increased with equilibrium concentration and was close to saturated amount of adsorption estimated from the CD content of the CDC beads (38.9 mg/g for NP and 40.3 mg/g for BPA) when the equilibrium concentration was larger than 10^{-4} mol/L.

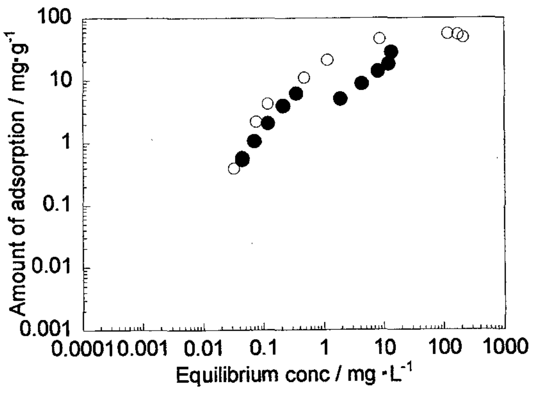


Figure 5 Adsorption isotherm of BPA (○) and NP(●) onto CDC beads as a Freundlich plot.

The data obtained with 10^{-4} mol/L solution were re-plotted in Figure 6 as a Langmuir plot. A relatively good linear relation was observed for both BPA and NP as in the case of our previous cotton-like CDC [5]. This also indicated, as described above, that the CD moieties on the CDC beads acted as adsorption sites. From the intercept on the y axis of regressions, it was possible to estimate the equilibrium amount of adsorption; 50 mg/g for 4-NP and 56 mg/g for BPA.

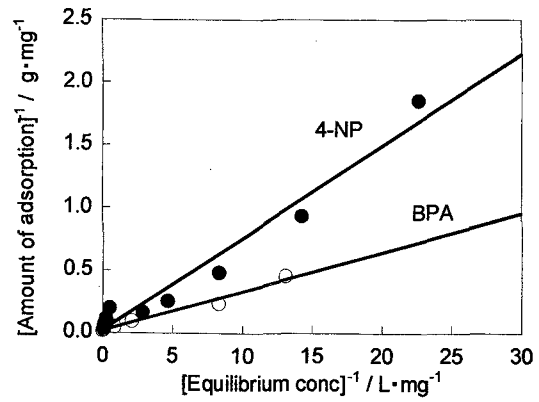


Figure 6 Adsorption isotherm of BPA (○) and NP(●) onto CDC beads as a Langmuir plot.

Alkylphenol ethoxylates are made from alkylphenols and used as industrially important nonionic surfactants. After released as a component of industrial waste water, the alkylphenol ethoxylates may be biologically or chemically degraded to the corresponding alkylphenols or their monoethoxylates which are suspected to be the endocrine disruptors. We investigated adsorption of various 4-nonylphenol ethoxylates (NPEs) onto the CDC beads. The alkylphenols could be removed by a polymer containing β-CD moieties [14]. We also carried out adsorption experiments by the CDC beads.

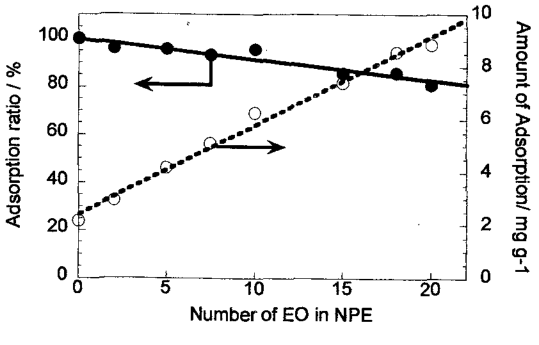


Figure 7 Adsorption of NPEs with various length of ethylene oxide chain.

Figure 7 shows the effect of number of ethylenoxide unit (EO) in NPE on the adsorption. The amount of adsorption, calculated using weight of adsorbed NPEs, increased with increasing of the number of EO. The molar amount of adsorption, however, clearly decreased with the number of EO. This can be attributed that the NPEs with longer EO chain are more hydrophilic so that the efficiency of adsorption by inclusion into the CD cavities became lower. In this experiment, even if the number of EO was 20, more than 80% of NPE was adsorbed.

4 CONCLUSION

Insoluble spherical chitosan beads bearing CD moieties were obtained by a condensation between multiply carboxymethylated β-CD and spherical chitosan beads under the alkaline conditions using water-soluble condensing agent. The CD content of the products reached 28% under the appropriate condition. Judging from the dissolution-deposition phenomenon observed under the mild conditions, the multiply carboxymethylated CD worked as crosslinking agent. The CDC beads adsorbed BPA, NP, and NPEs. The adsorption was attributed to the inclusion phenomena at the introduced CD moieties.

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