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Synthesis of Endocrine Disruptor Absorbents Based on Cycxlodextrin

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Two kinds of absorbents (**1** and **2**) for endocrine-disrupting chemicals have been synthesized from β-cyclodextrin, in which **1** is β-cyclodextrin polymer combined with rice husks ash of 112 m^2/g specific surface and **2** is β-cyclodextrin polyalkylene polyamine linked with polystyrene. Those absorbents are not soluble in water and exhibited removal capability for endocrine disruptor in aqueous layer with high efficiency. Absorbent **1** as well **2** show high selectivity for **4,4'**-isopyropidendiphenol (bisphenol A) with 97.15% and 98% removal rate respectively. On the other hand, β-cyclodextrin polymer, which is non-coexistent with rice husks ash and polystyrene itself show 63% and only 2% removal rate of bisphenol A respectively. It is useful in a combination between β-cyclodextrin and rice husks ash or polystyrene, however, the complex of β-cyclodextrin polymer combined with amorphous silica (**5**) is not useful, which exhibits less 60% removal rate of bisphenol A.

Key Words : absorbent, endocrine-disrupting chemical, β-cyclodextrin, rice husks ash

1. Introduction

The effects on endocrine-disrupting chemicals for humans, animals, and environments have been serious rekindling of interest in a great deal of public attention[1,2]. It has been defined that endocrine-disruptor can influence the endocrine system of life. In recent years, a growing body of scientific research indicates that substances in the environment may interfere with the normal function of the endocrine system of humans and wildlife. These compounds may be man-made, e.g., industrial chemicals, crop protection chemicals, or they may be natural like the phytoestrogens. Some scientists have hypothesized that minute amounts of these chemicals are able to disrupt the endocrine system and cause cancer, harm to male (e.g., reduced sperm counts) and female reproductive systems, and other adverse effects. It has been reported that over seventy chemicals such as polychlorinated biphenyl (PCB), dichloro diphenyl trichloroethane (DDT), nonyl phenol and bisphenol A have been suspected as endocrine disruptors[3]. A couple of absorbents which are able to clean up endocrine disruptors in aqueous layer have been reported[4]. It seems to be hard for those systems to show high selectivity for specific endocrine disruptors because the absorbent mechanism is based on adsorption effect. Cyclodextrins, torus-shaped cyclic oligomers of D-glucopyranose, are named α-, β-, and γ- for hexa, hepta and octamers respectively. They can make host-guest complexation with a variety of organic compounds through hydrophobic interaction. Over the past half decade, we investigated fluorescent sensing system for endocrine disruptors based on modified cyclodextrins with chromophores such as dansyl, anthranilate, and tosyl units[5], because cyclodextrin itself can accommodate with endocrine disruptors. Cyclodextrins are water-soluble materials, which is the problem when we use cyclodextrins as absorbents for endocrine disruptors because absorbents should be water insoluble materials. To overcome this problem, we synthesize new types of absorbents (**1** and **2**)[6,7], in which **1** is complex of β-cyclodextrin polymer and rice husks ash and **2** is a complex between tetraethylenepentamine modified β-cyclodextrin and polystyrene resin. In this contribution, we would like to describe the capability of cleaning up endocrine disruptors from aqueous layer by those water insoluble absorbent materials derived from β-cyclodextrin.

2. Experimental

2.1 Synthesis

Preparation of 1

To a sodium hydroxide aqueous solution (NaOH 4 g / H_2O 20 ml), β-cyclodextrin 7 g (6.167 mmol) and epichlorohydrin (2.28 g, 24.57 mmol) and rice husks ash (3 g) which has 112 m^2/g specific surface and 1.3% loss of ignition, were added. The reaction mixture was stirred at 80℃ for overnight. After cooling to the room temperature, the reaction mixture was neutralized with 2N-HCl aqueous solution to yield water insoluble precipitates. After filtered off and then washed with water, methanol and dimethylforamide sequentially and dried in vacuo to yield β-cyclodextrin rice husks ash epichlorohydrin hybrid polymer (**1**). Yield point: 5.64 g.

Preparation of 2

To a solution of β-cyclodextrin tosylate (1.00 g, 0.776 mmol)

in demethylformamide (10 ml), tetraethylenepentamine (2.94 g, 15.5 mmol) was added. The reaction mixture was heated at 80℃ for overnight. After cooling to the room temperature, the reaction mixture was poured into 300 ml of acetone. The resulting precipitates were filtered off and then washed with methanol and dried in vacuo to yield crude tetraethylene modified β-cyclodextrin (TEP-β-cyclodextrin). Yield point: 600 mg (59% yield).

To a suspension of 4-chloromethyl polystyrene (100 mg, Tokyo Kasei cat. No. C1643) in dimethylformamide (2 ml), 100 mg of TEP-β-cyclodextrin (100 mg) was added initially, and then 20 mg of TEP-β-cyclodextrin were added day by day. Total amount of TEP-β-cyclodextrin added was 200 mg. The reaction mixture was stirred at 80℃ for a week. After cooling to the room temperature, the precipitates were filtered off and then washed with water, methanol, dimethylformamide consecutively and dried in vacuo to yield polystyrene-TEP-modified-β-cyclodextrin (2). Yield point: 120 mg.

Preparation of β-cyclodextrin polymer (3)

Preparation of β-cyclodexrin epichlorohydrin polymer (**3**) was prepared according to previously reported procedure[8]. To a sodium hydroxide aqueous solution (NaOH 4 g / H_2O 20 ml), β-cyclodextrin 7 g (6.167 mmol) and epichlorohydrin (4.56 g, 49.3 mmol) were added. The reaction mixture was stirred at 80℃ for overnight. After cooling to the room temperature, the reaction mixture was neutralized with 2N-HCl aqueous solution. The obtained precipitates were filtered off from the solvent, washed with water, methanol, and dimethylforamide sequentially and dried in vacuo. Yield point: 4.5 g.

Preparation of β-cyclodextrin amorphous silica epichlorohydrin hybrid polymer (5)

To a sodium hydroxide aqueous solution (NaOH 4 g / H_2O 20 ml), β-cyclodextrin 7 g (6.167 mmol) and epichlorohydrin (2.28 g, 24.57 mmol) and amorphous silica (3 g) which has 20 m^2/g specific surface and 1.3% loss of ignition, were added. The reaction mixture was stirred at 80℃ for overnight. After cooling to the room temperature, the reaction mixture was neutralized with 2N-HCl aqueous solution to yield water insoluble precipitates. After filtered off and then washed with water, methanol and dimethylforamide sequentially and dried in vacuo to yield β-cyclodextrin amorphous silica epichlorohydrin hybrid polymer (**5**). Yield point: 5.30 g.

2.2 Measurements

The extraction rate by absorbents was measured with Shimazu SCL-6B of HPLC analysis. The extract was applied to a reverse phase-column (YMC-Pack ODS-AM AM303, 250x 4.6 mm I.D, S-5 mm, 120 Å). A methanol aqueous solution (75 vol.%) with 0.9 ml/min., was used as the eluant for HPLC analysis. The endocrine disruptor analogues were adjusted at 23.15 ppm in 10 vol.% methanol aqueous solution. Absorbents **1** and **2** were added to 10 ml of the solution including 23.15 ppm endocrine disruptor and stirred at room temperature for 7 hrs. The 0.5 ml of supernatant fluid was taken and filtered off with 0.4 mm ID membrane filter. The filtrate was applied for HPLC analysis. The extract ability of **1** and **2** were determined by means of the following equation:

Extractability (%) $= [[A_0 - A]/A_0] \times 100$

Where A_0 is the concentration of endocrine disrupting chemical (ppm), and A is the concentration of remaining endocrine disrupting chemical (ppm).

3. Results and Discussion

The absorbent **1** was synthesized from β-cyclodextrin, epichlorohydrin and rice husks ash in the presence of NaOH as shown in Figure 1. We previously reported the SEM images of absorbent (**4**)[9], which β-cyclodextrin polymer combined with rice husks ash. Figure 2 shows the images of **4** and **4'**, which was synthesized from **4** by heated at 600℃. The rice husks ash seems to act as support medium in resulting yielding amorphous fine pores of cyclodextrin polymer, which works as remover of endocrine disruptor analogues examined here with high efficiency. On the other hand, β-cyclodextrin polymer (**3**) and rice husks ash itself show 63% and 2% remove rates for bisphenol A respectively. This result suggested that the combined structure of β-cyclodextrin and rice husks ash can properly act as absorbent. Figure 3 shows that removal rates for eight endocrine disruptors, by **1**. As shown in Figure 3, bisphenol A was removed with most effectiveness and in sequence 1,2-dichlorobenzene, 2,4-dichlorophenol, 2,4,6-trichlorophenol, diethyl phthalate, 3,4-dichlorobenzoic acid, and 2,4-dichlorophenoxyacetic acid were removed by **1**. It is suggested that the removal capability of **1** for those guests are depended on the guest molecular size except of bisphenol A, because the CPK model for those guests show the real guest molecular sizes in order as shown in Figure 4. Why does **1** removes with bisphenol A with most effectiveness, nevertheless of biggest size of bisphenol A among the guest molecules exam-

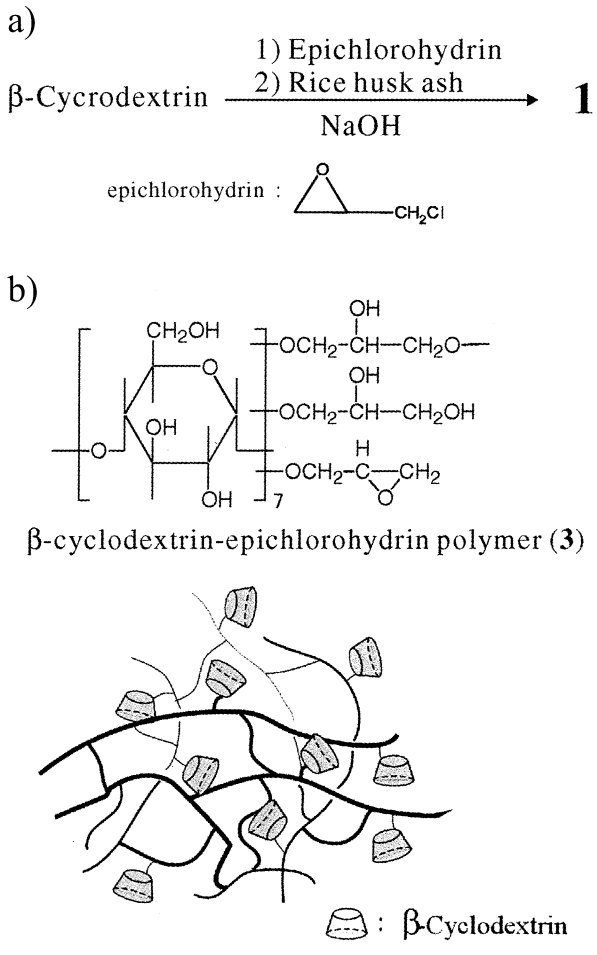
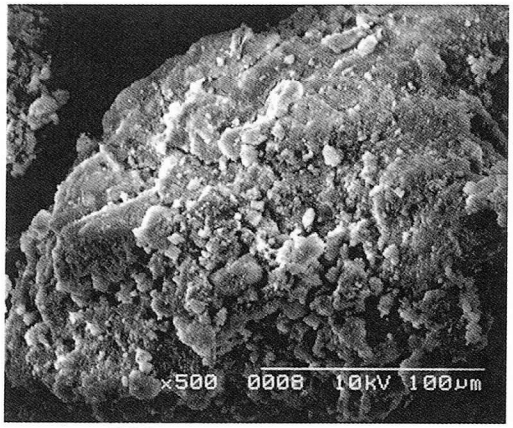
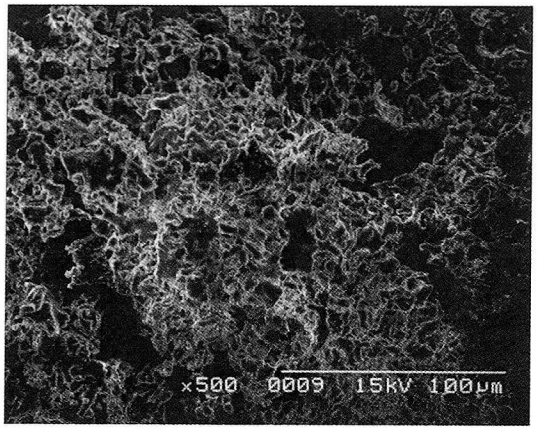


Figure 1 (a) Preparation of **1**. (b) The estimated structure of **3**.



x500 ——— 100 μ m

4



x500 ——— 100 μ m

4'

Figure 2 SEM images of **4** and **4'**.

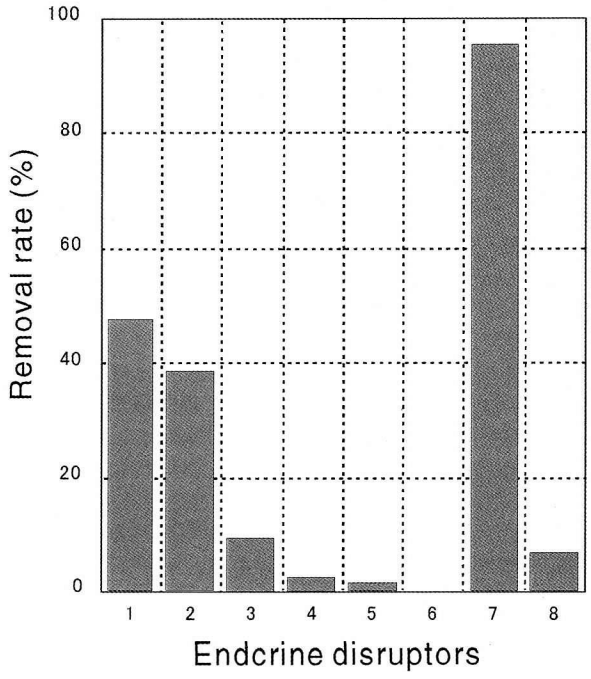


Figure 3 Removal rates of for endcrine disruptors by **1**.

ined. It is probably that there is some interaction such as hydrogen bonding between phenolic hydroxyl group and secondary hydroxyl group of β-cyclodextrin. It might be that guests **2** and **3** are too large to be included in the cyclodextyrin cavity because bulky group such as chlorine residue attached to the benzene ring. It is apparently that a part of phenol unit not whole part of bisphenol A should be included in the cyclodextrin cavity. Figure 5 shows that correlatively of the volume of adsorbent and removal rate for endocrine disruptors by **1**. Over 10 mg of **1** shows almost 100% removal rate for bisphenol A. Previously we reported that absorbent (**4**), which showed high removal capability for dibenzofuran with most efficiently. It is different result obtained from that of **1**, which is hard to work for dibenzofuran. In the system of **4**, the specific surface of rice husks ash and the mixed ratio of β-cyclodextrin and rice husks ash are different from those of **1**. It means that the specific surface of rice husks and the mixed ratio of rice husks ash and β-cyclodextrin have an influence on removal capability for endocrine disruptors. We also investigated the capability of **2** and **5** for bisphenol A. The capability of **2** shows 98% removal rate for bisphenol A. It is probably hydrophobic effect existing because the polystyrene units should provide hydrophobic domain, which increase hydrophobic interaction between β-cyclodextrin and bisphenol A. On the other hand, the removal

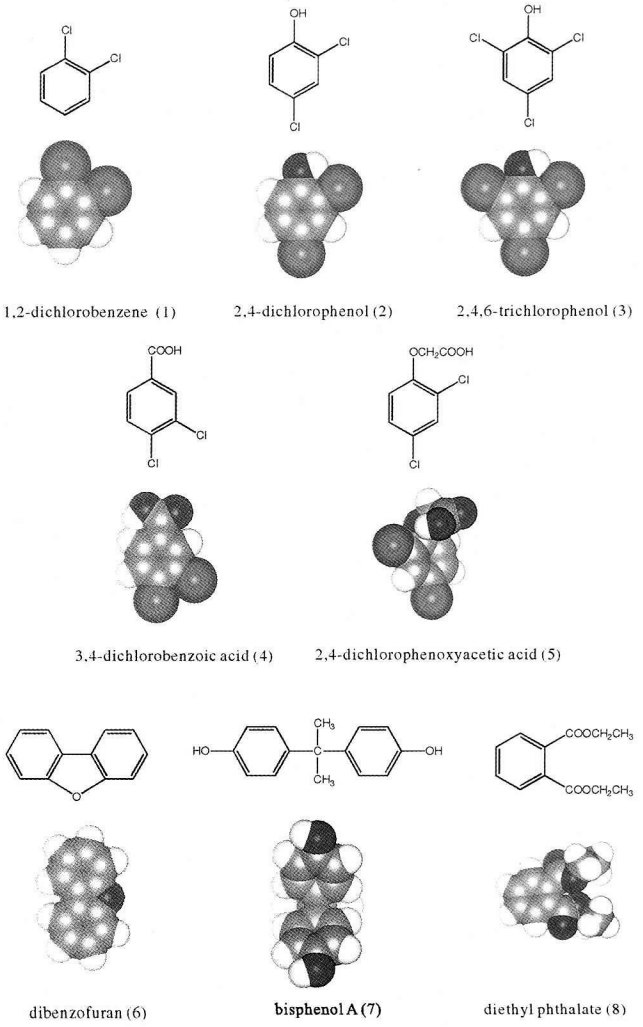


Figure 4 Endcrine disruptors and those CPK models.

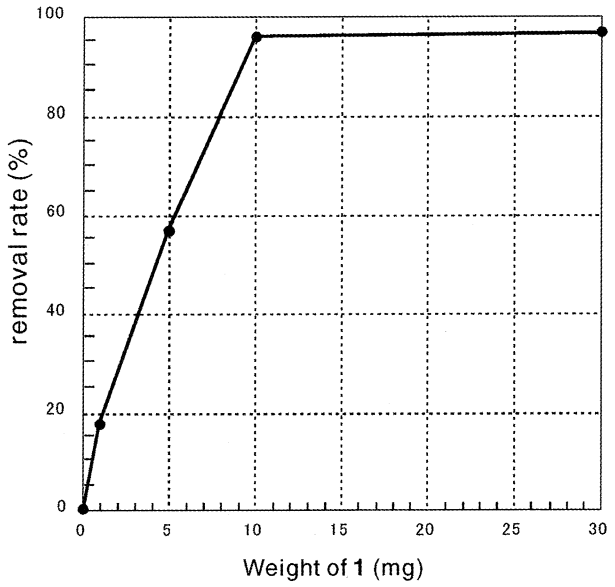


Figure 5 Removal rate of bisphenol A depend on **1** quantity (Bisphenol A: 0.1 mM/10 vol.% MeOH aqueous solution (10 ml)).

of **5** shows less 60% rate for bisphenol A, which is almost same as that of **3**. It is indicated that the effect of amorphous silica is hard to be recognized as an efficient absorbent in this system. Point of view of utilization of industrial and trade waste products, it seems to be important to reuse rice husks, which are in production about 2,320,000 ton per a year. Unfortunately, effective use method is no realized. In conclusion, the effective usage of rice husks ash will contribute a remediation of environment.

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