Adsorption of Di-*n*-butyl Phthalate by Chitosan Beads Modified with Water-soluble Calixarenes

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(Received November 24, 2004; CL-041415)

The binding ability of di-*n*-butyl phthalate (**DBP**) by *p*-sulfonatocalix[*n*]arenes {**SCA*n*** ($n = 4, 6, 8$)} and adsorption ability of **DBP** by chitosan beads modified with **SCA*n*** were investigated. **SCA6** and **SCA8** could include **DBP** in their hydrophobic cavities in aqueous phase. The amount of **DBP** adsorbed by chitosan beads modified with **SCA6** was approximately 5 times as large as that for unmodified chitosan beads.

Endocrine-disrupting chemicals (EDCs) and persistent organic pollutants have received much attention in recent years. Nonylphenol, 4-octylphenol, and bisphenol A have been reported to show estrogenic activity,[1–3] and various other chemicals are suspected of being EDCs. Alkyl phthalates are representative examples,[4,5] and are still under investigation. These chemicals have been detected in rivers and sediments because of their widespread use as plasticizers. Activated carbon has generally been used as the adsorbent for organic pollutants in water. However, the breakthrough time of activated carbon is not long, because the selectivity for adsorbates is not sufficient, and thus reactivation processing of the used activated carbon is frequently necessary. In addition, a large amount of thermal energy is required for the reactivation processing. If an adsorbent with recognition ability could be prepared, these problems could be solved. To this end, several studies have reported adsorbents with molecular recognition sites, e.g., the adsorbent with cyclodextrin adsorbed bisphenol A and alkyl phthalates.[6–8] If the receptor has a hydrophobic cavity formed by an aromatic ring, strong interaction is expected between the receptor and the noxious hydrophobic chemicals. The authors propose an adsorbent modified with calixarene, which is a cyclic oligomer that consists of aromatic rings crosslinked by methylene groups, as a binding site. The backbone of this adsorbent is chitosan, an environmentally friendly hydrophilic polymer derived from the discarded shells of crabs and shrimp. In this paper, we describe the adsorption ability of di-*n*-butyl phthalate (**DBP**) by chitosan beads modified with *p*-sulfonatocalix[*n*]arenes (**SCA*n***) in an aqueous system and the inclusion properties of **DBP** by **SCA*n***.

Calix[*n*]arenes ($n = 4, 6, 8$) are known that can be easily synthesized from *p*-alkylphenol and formaldehyde with good yields.[9] From a practical viewpoint, it is appropriate that these analogues are nominated as a basic skeleton for **DBP** adsorbent. In addition, it is important that the fabrication of the adsorbent is convenient for industrialization. Polyion complexation is one kind of convenient modification method for polymer. Immobilization of anionic sulfonated calixarenes could be achieved by a polyion complex formation with the cationized chitosan. Thus, we synthesized **SCA*n*** ($n = 4, 6, 8$) and 2,6-dimethyl sulfonatophenol (**DMSP**) as reference (Figure 1). **SCA*n*** ($n = 4, 6, 8$) were synthesized by the reported method.[10] **DMSP** was prepared by direct sulfonation of 2,6-dimethylphenol using the same method as for **SCA*n***.

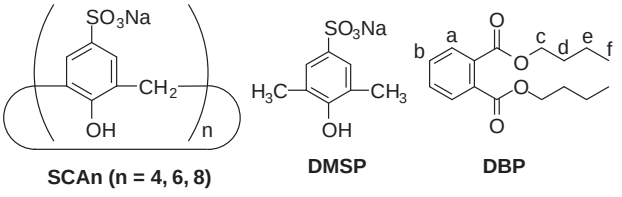

Figure 1. Chemical structures of **SCA*n***, **DMSP**, and **DBP**.

Table 1. Ratio of **SCA*n*** ($n = 4, 6, 8$) (or **DMSP**) to chitosan in adsorbent determined by XPS

Receptor	S_{2p} (in receptor)/ N_{1s} (in chitosan)	Chitosan unit: receptor
DMSP	0.47 ($\sigma^a = 0.02$)	100:47
SCA4	0.65 ($\sigma^a = 0.03$)	100:16.3
SCA6	0.59 ($\sigma^a = 0.04$)	100:9.8
SCA8	0.63 ($\sigma^a = 0.04$)	100:7.9

[a]σ is a standard deviation calculated from 20 data.

SCA*n* and **DMSP** were immobilized onto chitosan beads by polyion complexation so as to fabricate the adsorbent with the molecular receptors.[11] Many types of chitosan beads are now commercially available. In this study, we chose Chitopearl Basic AL-01 (**AL-01**), which is manufactured by Fuji Spinning Co., Ltd. (Tokyo, Japan). **AL-01** is unsubstituted chitosan beads with a particle diameter of 74–210 μm. Anionic **SCA*n*** and **DMSP** were complexed onto the cationized **AL-01** in acetic acid solu-

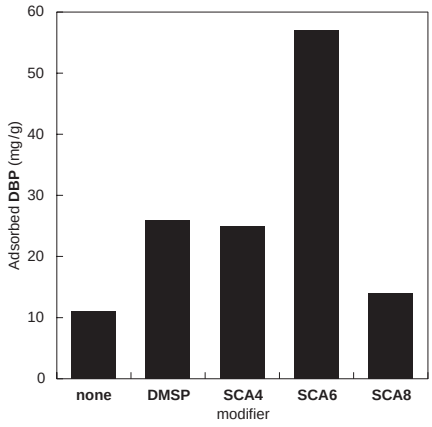

Figure 2. Adsorption of **DBP** by chitosan beads modified with **SCA*n*** or **DMSP**.

tion. The ratios of molecular receptor to chitosan unit were estimated by X-ray photoelectron spectroscopy (XPS). The results are shown in Table 1. The S_{2p}/N_{1s} ratio was approximately 0.5 in all molecular receptors. If the conformational structures and hole size of **SCA*n*** are not considered, then the hydrophobicities of the prepared chitosan beads were almost the same in the case of **DMSP**.

Adsorption tests for **DBP** on the chitosan beads modified with **SCA*n*** (**SCA*n*-C**) or **DMSP** (**DMSP-C**) were carried out in 300 mL Erlenmeyer flasks. One gram of **SCA*n*-C** or **DMSP-C** and 100 mL of 67 mM phosphate buffer (pH 7.0) containing 12 mM of **DBP** were placed in the flask and shaken for 24 h at 298 K. The adsorbed **DBP** was extracted in *n*-hexane and detected by a UV spectrophotometer. The results are shown in Figure 2. The chitosan beads adsorbent modified with **SCA6** had approximately 5 times the adsorption ability of unmodified chitosan beads. The chitosan beads modified with the other receptors did not show such a large improvement in adsorption.

In order to investigate the mechanism of the adsorption, the complexation behavior of **SCA*n*** or **DMSP** as a molecular receptor with **DBP** was examined by ^{1}H NMR measurement as follows (400 MHz, $T = 298 \pm 2$ K).[12] To 2.5–124 mM of **SCA*n*** or **DMSP** solution in D_2O, stock methanol-d_4 solution of **DBP** was added. At this time the concentration of **DBP** was kept constant at 1.2–1.3 mM and the host–guest solution contained 0.8% of methanol-d_4 from stock solutions. When **SCA6** or **SCA8** was used as a receptor, association constants (K_a) were calculated using a non-linear curve fit of the aromatic signals of **DBP**.[13]

When **DMSP** or **SCA4** was used as a molecular receptor, the chemical shift of **DBP**'s protons did not shift to a higher magnetic field (Figures 3a and 3b). These results indicated that neither **DMSP** nor **SCA4** included **DBP**. In contrast, the signals attributed to the aromatic protons and the alkyl protons of **DBP** were shifted to a higher magnetic field in the presence of **SCA6** (Figure 3c). These results were caused by the magnetic shield effect of the aromatic rings of the host. This means that **SCA6** could include **DBP** in its cavity ($K_a = 215\ M^{-1}$). The same tendency was observed in the case that **SCA8** was used as a molecular receptor (Figure 3d). Therefore, **SCA8** could bind **DBP** in aqueous phase ($K_a = 372\ M^{-1}$). The binding ability of **SCA8** to **DBP** was stronger than that of **SCA6** owing to the flexible skeleton. Although **SCA4** has a hydrophobic cavity like **SCA6** and **SCA8**, the ^{1}H NMR chemical shift of **DBP** did not change. The reason **SCA4** showed no inclusion of **DBP** in its cavity is that **SCA4** has a smaller cavity and more rigid skeleton than **SCA6** or **SCA8**.

SCA8 showed stronger binding ability to **DBP** than **SCA6** did in homogeneous aq solution, but the chitosan beads modified with **SCA8** showed poor adsorption ability. This was probably due to the difference of the ring size and the flexibility between **SCA6** and **SCA8**. The ring size and the flexibility of molecular receptors play an important role in complexation between **DBP** and a free molecular receptor. A large ring on the host would lead to easy insertion of a guest, and the flexible structure would change the conformation for suitable hydrophobic interaction between the host and guest. On the other hand, larger ring size and greater flexibility, which means a large number of units, might cause destruction of the hydrophobic cavity by immobilization onto chitosan beads. Because **SCA8** has eight sulfonate groups that can link to the amino groups in chitosan, anchoring in many ways and a large number of anchorings would limit the conformational changes of **SCA8**.

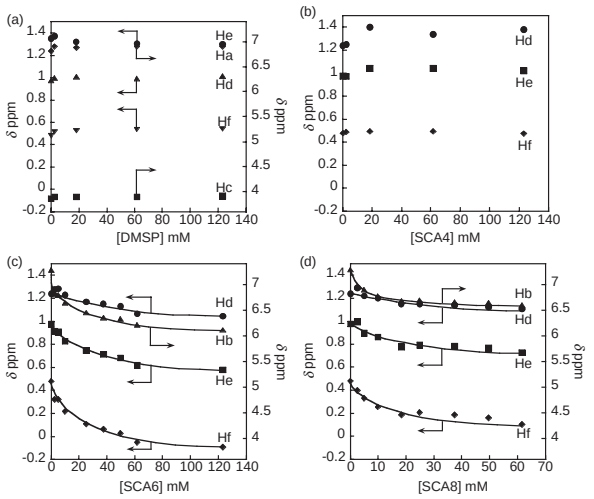

Figure 3. ^{1}H NMR chemical shift (in ppm) of **DBP** in D_2O (with 0.8% of CD_3OD) in the presence of (a) **DMSP**; (b) **SCA4**; (c) **SCA6**; (d) **SCA8**.

In conclusion, chitosan beads with **SCA6** were shown to adsorb **DBP** as contrast to chitosan beads with **SCA8**, even though **SCA8** has a stronger binding ability to **DBP** than **SCA6** did in aq solution. These results suggest that the steric complementarity should be kept on the immobilization of molecular receptor onto support.

This work was partially supported by "Nanotechnology Support Project" of the Ministry of Education, Culture, Sports, Science and Technology (MEXT), Japan.

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Published on the web (Advance View) January 15, 2005; DOI 10.1246/cl.2005.218