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Polymer-based adsorption medium prepared using a fragment imprinting technique for homologues of chlorinated bisphenol A produced in the environment

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Abstract

A polymer-based adsorption medium having molecular recognition ability for homologues of chlorinated bisphenol A produced in environment was prepared using a fragment imprinting technique. 2,6-Dimethyl phenol was utilized as a pseudo-template molecule and the adsorption media prepared was evaluated by high performance liquid chromatography (HPLC) and solid-phase extraction (SPE). As results, the adsorption medium showed preferable chromatographic retention and specific adsorption ability for the chlorinated bisphenol As having chlorine substituents at 3,5-positions through fragment imprinting effect.

Keywords: Fragment imprinting; Molecular recognition; Polymer-based adsorbents; Endocrine-disrupting chemicals; Bisphenol A

1. Introduction

To achieve easy and effective analysis of environmental organic as well as inorganic substances, pretreatment using adsorbent having specific molecular recognition ability is quite useful and important [1,2]. Such specific molecular recognition ability can be realized with molecularly imprinted polymer (MIP). MIP shows specific molecular recognition ability for the target molecule utilized as template molecule through imprinting effect [3].

In the usual or traditional molecular imprinting method for some organic compound, cross-linking agent, template molecule, polymerization radical initiator, and functional monomer, which can interact with the template through non-covalent type molecular interactions, such as hydrogen bonding, ionic interaction, and/or hydrophobic interaction, are polymerized at an elevated temperature all together. The polymer prepared obtains specific recognition sites for the template through imprinting effects. Since molecular imprinting method is rather easy method, MIP is used as various media such as artificial antibody as well as stationary phase for high performance liquid chromatography (HPLC) [4].

Again, molecular imprinting method should require template molecule directly to get specific molecular recognition ability. However, it becomes a serious problem, if we have to obtain the molecularly imprinted, specific molecular recognition ability towards some toxic compound or very rare compound [5].

Moreover, when we utilized the template molecule, it cannot be removed completely from the polymer prepared even after tedious repeated washing process with appropriate organic solvents, because the imprinted sites can be also formed not only on the surface but also deeply in the cross-linked polymer network structure, where organic solvent can hardly reach. This can be the other serious problem in trace analyses of environmental compounds by gradually leaked template molecule from the polymer matrix, when we have to determine even ppt level of those compounds.

The substituted compounds by –OH group and –Cl group are easily formed in human body and in environment, respectively. The number of substituents as well as their substitution positions cannot be easily expected, therefore, usual

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molecular imprinting method does not make any sense. We are trying to establish a new strategy to prepare selective molecular recognition media using novel fragment imprinting technique [6].

Chlorinated bisphenol A (Cl-BPA), homologue of bisphenol A (BPA) was firstly reported in "Tagonoura" fleet in Shizuoka prefecture in Japan [7]. These are produced during bleaching processes of recycled paper in paper factories and several homologues based on the number of chlorine substituents and their substitution positions have been confirmed.

The strength as endocrine disruptor chemicals has been tested using two different processes, and majority of chlorinated bisphenol As (Cl-BPAs) have stronger toxicity than no-substituted BPA [8]. In addition, no-substituted BPA was found to be easily decomposed biologically in environmental water, however, Cl-BPAs become resistant to the biological decomposition due to chlorine substituents.

For quantitative analysis of toxic compounds in environment, solid-phase extraction (SPE) is the most important pre-treatment method because the concentration of those compounds is usually quite low and a lot of contaminations are coexisting in environment. However, the majority of commercially available SPE adsorbents (e.g. C_{18} cartridge), has relatively wide selectivity and can hardly work toward a specific molecule effectively with removal of other chemically similar contaminants. Therefore, a novel adsorbent having selective adsorption ability for certain toxic substance is required.

In this paper, we wish to report a new strategy to get selective molecular recognition ability for homologues of an environmental toxic compound, BPA through a preparation of the polymer without BPA as the direct template molecule. We wish to evaluate the adsorption ability of the polymer for Cl-BPAs. For this purpose, uniformly sized polymer particles were prepared using 2,6-dimethyl phenol (DMP) as a pseudo-template [9–12] through the two-step swelling and polymerization method [13] to examine the adsorption ability for Cl-BPAs reported as a stronger endocrine disruptor chemical from environmental water than BPA.

2. Experimental

2.1. Materials

Monomers, ethylene glycol dimethacrylate (EDMA) as a cross-linking agent, and 4-vinylpyridine as a functional monomer, both from Wako Chemicals (Osaka, Japan) were purified by vacuum distillation techniques to remove polymerization inhibitor [14]. Template molecules, *p-tert*-butylphenol (TBP) and 2,6-dimethyl phenol (DMP), and BPA were purchased from Nacalai Tesque (Kyoto Japan) and used as received. A polymerization radical initiator, 2,2′-azobis-(2,4-dimethyl-valeronitrile) (ADVN) was purchased from Wako Chemicals (Kyoto, Japan) and purified using a standard purification method. A solvent realizing porous structure (porogenic solvent), toluene from Nacalai Tesque was of the highest grade and used as received.

2.2. Preparation of the molecular imprinting polymer

To prepare polymer-based separation media, we utilized two-step swelling and polymerization method, which afforded uniformly sized polymer particles, utilizing polystyrene seed particles as shape templates [15,16]. The polystyrene seed particles were prepared through an emulsifier free emulsion polymerization, which has been reported elsewhere [17].

The two-step swelling and polymerization method easily afforded uniformly sized polymer particles nicely with the following feed ratio; EDMA: 10.0 ml, 4-vinylpyridine as functional monomer: 0.992 ml, toluene: 10.0 ml, TBP (0.173 g) or DMP (0.140 g), ADVN: 0.7 g (EDMA/4-vinylpyridine/TBP (or DMP) = 46/8/1, in mole ratio). The polymerization was carried out at 50 °C for 24 h.

The symbols of the polymers prepared using TBP and DMP are E-tol–TBP–MIP and E-tol–DMP–MIP, respectively. We also prepared the polymer without any template molecule (blank polymer) abbreviated as E-tol. The molecular structures of templates are depicted in Fig. 1.

The polymer particles prepared were dispersed into methanol and the supernatant was discarded after sedimentation of the polymer particles. This procedure was repeated three times in methanol and twice in tetrahydrofuran (THF), and then the polymer particles were filtered with a membrane filter and dried at room temperature to determine the chemical yields. This process can also realize removal of the pseudo-template molecules from polymer particles. The chemical yields were almost quantitative [18]. The polymer particles had 10.4 μm in diameter and the size uniformity of the polymer particles was excellent as reported previously [18], which is quite important for smooth treatment of environmental water samples including lots of contaminants.

2.3. Preparation of Cl-BPAs

The preparation method was reported in the previous paper [7]. 6.85 g (30 mmol) of BPA was dispersed in 70 ml of methanol. An aqueous solution (5%) of NaClO (100 ml) was added into the dispersion and the chlorination reaction took place for 10 h at room temperature with slow stirring.

After the reaction, 50 ml of sodium sulfate aqueous solution (1 M) was added into the solution and methanol was removed under reduced pressure. Finally, aqueous hydrochloric acid (10%) was added and the products were extracted with dichloromethane.

The organic layer was washed with brine and dried over anhydrous magnesium sulfate. After removal of dichloromethane, yellow paste (4.13 g) was obtained. The paste included homologues of Cl-BPAs and determined by HPLC–MS technique. The mixture of homologues were separated

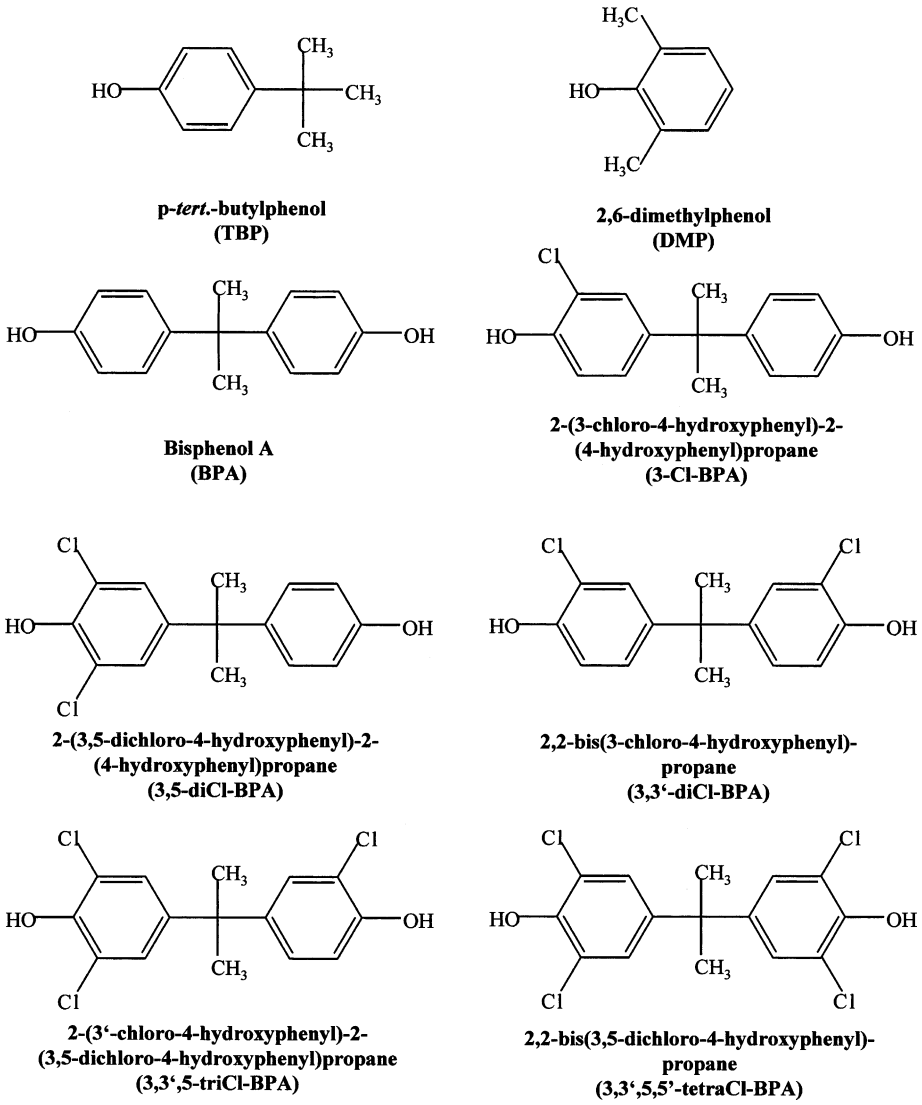

Fig. 1. Structure of a pseudo-template molecule and Cl-BPAs.

from each other by silica gel column chromatography using mixture of chloroform and methanol as mobile phases. The molecular structures of Cl-BPAs are depicted in Fig. 1.

3. Results and discussions

3.1. Homologues of chlorinated BPA

As shown in Fig. 2a and b, the yellow paste obtained includes mono-, di-, tri-, and tetra-chlorinated BPAs. In this case, observed peak height does not directly mean the content of compounds. If we carefully check, at least two isomers of di-substituted Cl-BPA were found to be obtained in the synthesis. This is an unexpected result because, the previous paper did not mention this phenomenon [7]. Through careful NMR determinations, two di-substituted Cl-BPAs were identified to be 3,5-diCl-BPA and 3,3′-diCl-BPA.

As mentioned in Section 2, we utilized DMP as a pseudo-template molecule. Through our previous studies, methyl substituent on phenyl ring is a possible mimic for chlorine substituent [5]. Therefore, the separation medium prepared in this study will recognize Cl-BPA having chlorine atoms at 3- and 5-positions of BPA's phenyl ring.

To examine about the effect of the pseudo-template molecule, the separation media were evaluated by HPLC. As shown in Table 1, imprinting effect for each template or structurally close molecules was observed. Moreover, as shown in Fig. 3, the separation medium prepared using DMP as pseudo-template molecule (E-tol–DMP–MIP) showed increments of relative retention α (k' solute/k' phenol) for 3,5-diCl-BPA as well as 3,3′,5-triCl-BPA compared those on the blank polymer (E-tol) and even those on the polymer prepared using TBP as template molecule (E-tol–TBP–MIP). Both homologues having larger α values have chlorine substituents at 3- and 5-positions of BPA's

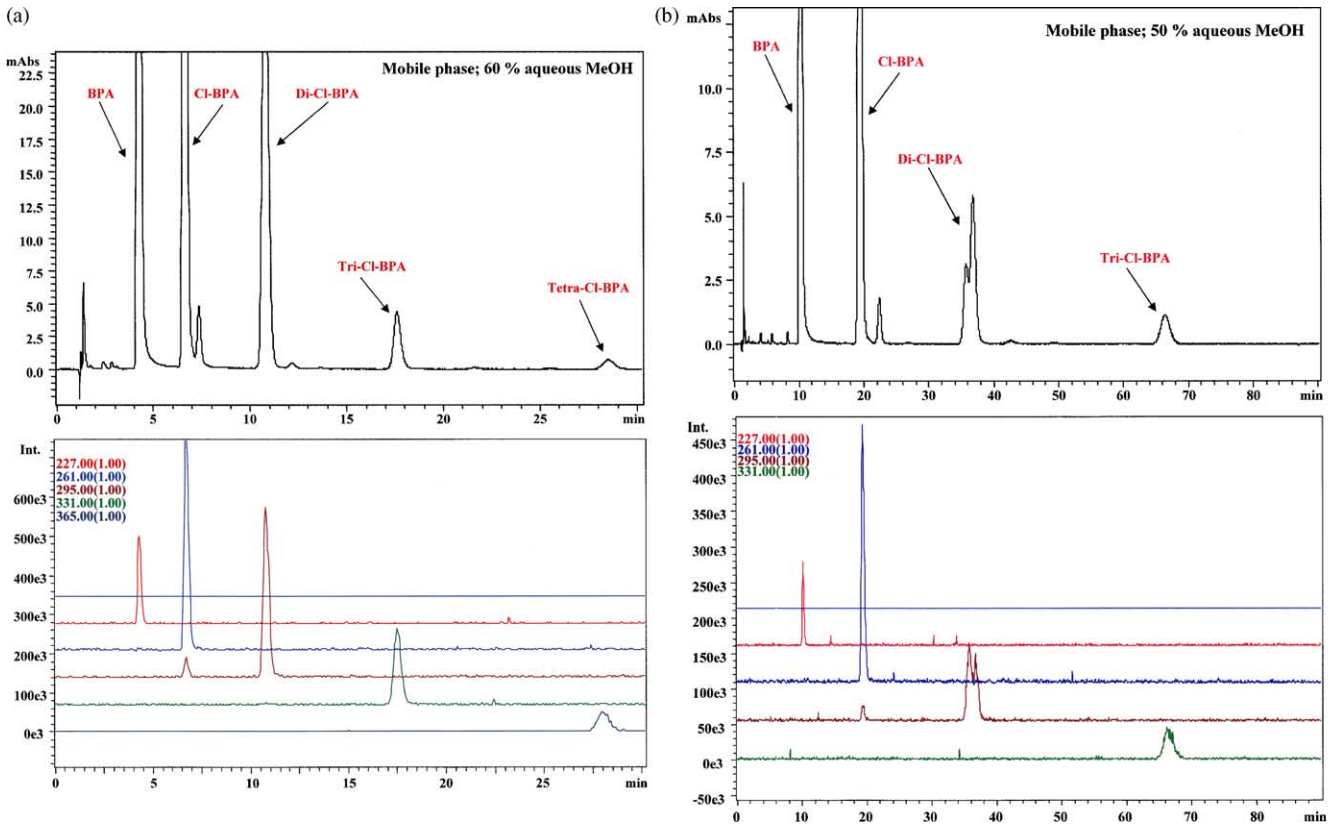

Fig. 2. HPLC–MS determinations of Cl-BPAs prepared. HPLC conditions: column; Mightysil (100 mm × 2 mm i.d.), mobile phase; 60% aqueous methanol (a) and 50% aqueous methanol (b), flow rate; 0.2 ml/min, detection; UV 280 nm, MS-SIM mode, temperature, 40 °C.

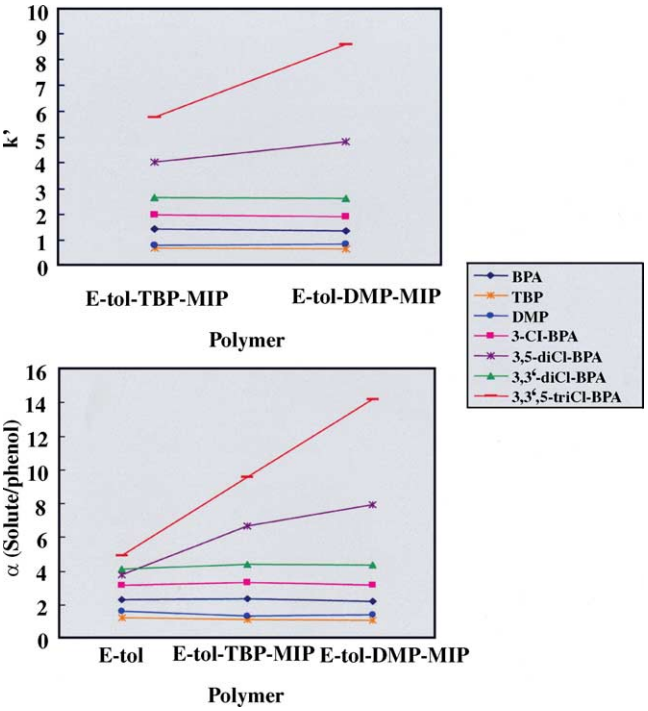

Fig. 3. Comparison of selectivity using k' and α values on polymers prepared. HPLC conditions were same as those described in Table 1.

phenyl ring. Interestingly, molecular recognition effect observed with the polymer prepared with pseudo-template molecule was more notable than those of the real template molecule.

We examined the adsorption performance of the prepared separation media; each separation medium (0.36 g) was packed into glass cartridge. The experimental procedure is as follows; 1000 ml of a dilute solution of BPA and Cl-BPAs was put through each cartridge (in this case, C_{18} silica-based adsorbent was also employed). After this process, 5 ml of methanol was flowed through the cartridge and the eluent from cartridge was collected every 1 ml. This

Table 1
Comparison of k' on each separation media

	E-tol	E-tol–TBP–MIP	E-tol–DMP–MIP
o-Cresol	0.530	0.721	0.738
m-Cresol	0.445	0.628	0.636
p-Cresol	0.433	0.622	0.629
BPA	0.937	1.413	1.322
TBP	0.499	0.662	0.618
DMP	0.642	0.800	0.814

HPLC conditions: mobile phase; 90% aqueous methanol, size of the columns packed with polymers prepared; 150 mm × 4.6 mm i.d., flow rate; 1.0 ml/min, Detection; PDA (280 nm), temperature; ambient.

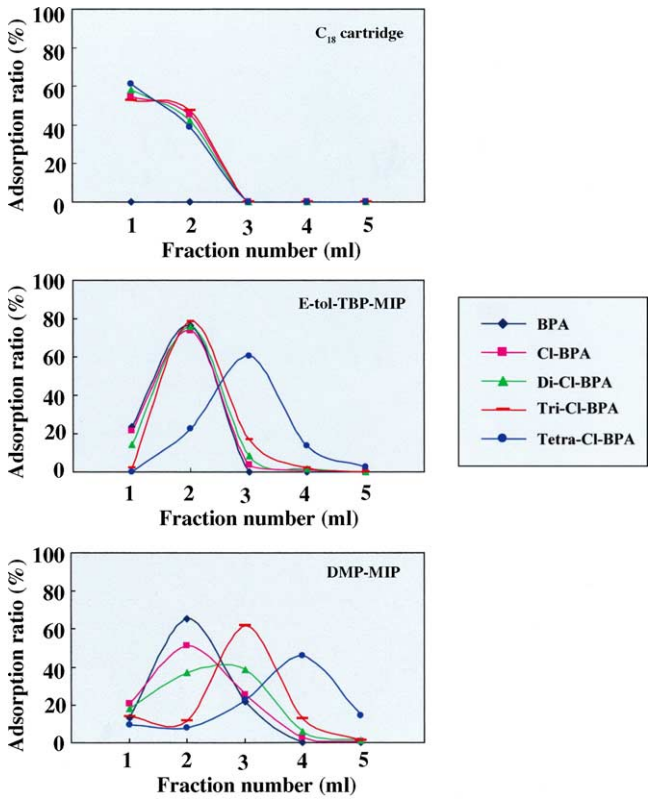

Fig. 4. Contents of fractions from the cartridges packed with adsorbents. Elution solvent; 50% aqueous methanol. Each fraction was tested independently and calculated the contents of Cl-BPAs. HPLC conditions for the determination of the contents were the same as those described in Fig. 2a.

elution process was repeated for five times (fraction number 1–5), and each fraction was defined by HPLC–MS method.

As schematically depicted in Fig. 4, on the cartridge packed with the polymer using DMP as the pseudo-template showed much longer retention for tri- and tetra-chlorinated Cl-BPAs compared with C_{18} cartridge as well as that prepared with TBP as the template. These findings suggest that some template effect with DMP plays an important role to adsorption for Cl-BPAs. Interestingly, even with relatively simple elution method described above, Cl-BPAs having different number of chlorine substituents could be roughly separated, while usual C_{18} cartridge could not separated at all.

4. Conclusion

A simple preparation method using a pseudo-template, 2,6-diemthylphenol realized selective recognition for chlorinated bisphenol A having the chlorine substituents at 3,5-positions. Through an adsorption-desorption method with the adsorption cartridge packed with polymer prepared with 2,6-dimethylphenol as a pseudo-template, chlorinated bisphenol A having different number of chlorine substituents could be separated, while usual C_{18} cartridge could not separated at all. This concept will be applied for other chlorinated bisphenol A having chlorine substituents at different position of bisphenol A if appropriate pseudo-template is utilized. The further studies are under progress.

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