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On-column concentration of bisphenol A with one-step removal of humic acids in water

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Abstract

An efficient extraction method for bisphenol A from environmental water including contaminants was developed using surface selective localization of functional group, on a polymeric separation device. The polymer utilized in this study was prepared through a kind of molecular imprinting technique, namely fragment imprinting effect utilizing a pseudo-template molecule (*p-tert.*-butylphenol) instead of bisphenol A. The concentration of bisphenol A onto the polymer device prepared, up to 1000 times concentration from environmental water including contaminants (humic acids), was achieved very easily with interesting exclusion effect for humic acids. The results obtained in this study suggest that molecular imprinting with the pseudo-template molecule is quite an effective way for selective concentration of the diluted target molecule from other contaminants including similar functional group with the target molecule.

Keywords: Water analysis; Endocrine disruptors; Molecular imprinting; Humic acids; Bisphenol A; Butylphenol; Phenols

1. Introduction

To achieve effective analysis of environmental organic as well as inorganic substances, adsorbents having specific molecular recognition ability are quite useful [1,2]. The specific molecular recognition ability can be easily realized with a specially prepared polymer adsorbent, namely a molecularly imprinted polymer (MIP). An MIP is prepared using a target molecule as the template molecule, and shows specific molecular recognition ability for the target molecule through imprinting effect (molecular imprinting method) [3].

In the usual or traditional molecular imprinting method for certain organic substances, the 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 all together at elevated temperature. The polymer prepared obtains a specific recognition site for the template through imprinting effects. Since the molecular imprinting method is a rather easy method, MIPs are used as various media such as artificial antibodies as well as stationary phases for high-performance liquid chromatography (HPLC) [4].

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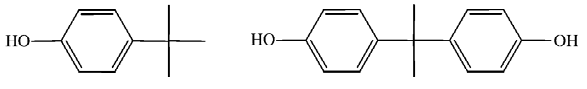
Again, the molecular imprinting method should require the template molecule to directly realize specific molecular recognition ability towards the template molecule. However, it becomes a serious problem if we have to obtain the molecularly imprinted specific molecular recognition ability for some toxic or very rare compound [5]. Moreover, the it is very difficult to completely remove the utilized template molecule from the prepared polymer even after a tedious repeated washing process with some organic solvents, because the imprinted sites can be formed not only on the surface but also deep in the cross-linked polymer network structure, where the organic solvent can hardly reach. This can be the other serious problem for trace analyses of environmental toxic compounds because we need information down to the ppt level.

In this paper, we report a possible method to achieve specific molecular recognition ability for an environmental toxic compound, bisphenol A, through preparation of the polymer without bisphenol A as the direct template molecule. We wish to evaluate the adsorption ability of the polymer for bisphenol A. For this purpose, uniformly sized polymer particles were prepared using *p-tert.*-butylphenol as a pseudo-template [6–10] through the two-step swelling and polymerization method [11] to examine the adsorption ability for bisphenol A, known as an endocrine disruptor chemical from environmental water. The target molecule, bisphenol A as well as the pseudo-template, *p-tert.*-butylphenol, are depicted in Fig. 1.

We also examine on-column removal of humic acids, which are contaminants in environmental water, during the adsorption process for bisphenol A based on a mechanism of ‘‘surface selective localization effect of functional group’’ through a molecular imprinting method, for selective 1000-fold concentration of bisphenol A from environmental water, which may realize quite easy ppt level analysis for bisphenol A in environmental water using HPLC system even with UV detector.



p-*t*-butylphenol bisphenol A

Fig. 1. Chemical structures of *p-tert.*-butylphenol and bisphenol A.

2. Experimental

2.1. Materials

Monomers, ethylene glycol dimethacrylate (EDMA) as the cross-linking agent, and 4-vinylpyridine as the functional monomer, both from Wako Pure Chemicals (Osaka, Japan) were effectively purified by vacuum distillation techniques to remove polymerization inhibitor [12]. Template molecules, *p-tert.*-butylphenol and bisphenol A 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 (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 devices, we the utilized two-step swelling and polymerization method, which afforded uniformly sized polymer particles, utilizing polystyrene seed particles as shape template [13,14]. The polystyrene seed particles were prepared through an emulsifier free emulsion polymerization, which has been reported elsewhere [15].

The two-step swelling and polymerization method easily afforded uniformly sized polymer particles with the following feed ratio: EDMA: 10.0 ml, 4-vinylpyridine as functional monomer: 0.992 ml, toluene: 10.0 ml, *p-tert.*-butylphenol: 0.173 g, ADVN: 0.7 g (EDMA–4-vinylpyridine–*tert.*–butylphenol, 46:4:1, in mole ratio). Polymerization was carried out at 50 °C for 24 h.

The prepared polymer particles 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. The chemical yields were almost quantitative [16]. The polymer particles were 10.4 μm in diameter. The size uniformity of the polymer particles was excellent as reported previously [16].

2.3. Concentration of bisphenol A

We prepared a water solution of bisphenol A including excess of humic acids as contaminant. The water solution contained bisphenol A (2 ppm), while humic acids were saturated in the water solution.

First, the prepared polymer particles (0.5 g) were packed into a glass cartridge having a syringe shape followed by water flow as a pre-treatment of the polymer adsorbent layer. Second, the prepared water solution was continuously pumped into the glass cartridge packed with the polymer adsorbents (~300 ml), followed by pure water (10 ml). Third, methanol was pumped into the glass cartridge to recover the adsorbed bisphenol A. Finally, the concentration of bisphenol A recovered from the glass cartridge was determined by HPLC with C_{18} column.

3. Results and discussion

To examine the adsorption phenomenon for humic acids on the polymer particles with *p-tert.*-butylphenol as the template molecule, first we utilized an inert column made from polyether ether ketone (PEEK) packed with the polymer particles prepared (MIP), because a stainless steel column possibly adsorbs humic acids.

The PEEK column in HPLC system was evaluated with 100% methanol or pure water as the mobile

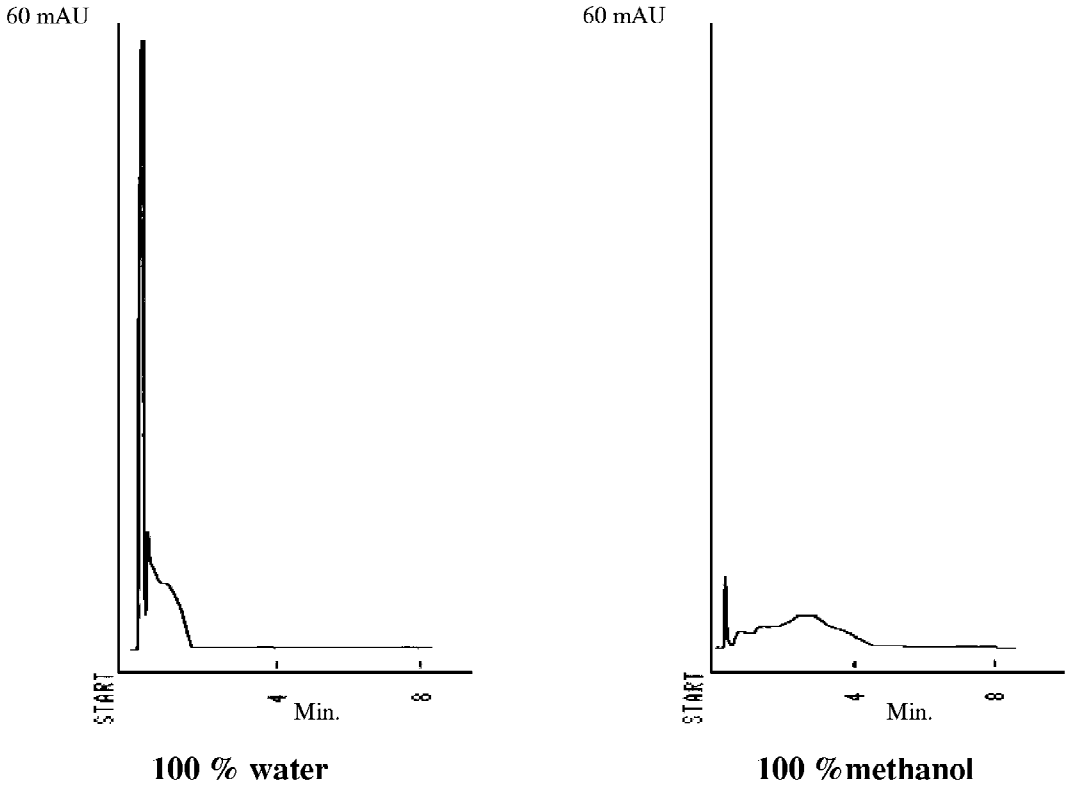


Fig. 2. Chromatograms of humic acids through the prepared polymer particle packed column with 100% water or 100% methanol. HPLC conditions: flow rate, 1.0 ml/min; column size, 30 mm×4.6 mm I.D. (PEEK); detection, UV 254 nm; temperature, 30 °C.

phase using commercial humic acids as solutes. The adsorption phenomenon of humic acids is shown in Fig. 2 as chromatograms obtained through HPLC evaluation.

As shown in Fig. 2, humic acids were eluted with very small elution volume both in methanol and water mobile phases. Furthermore, in the water mobile phase, the amount of eluted humic acids was greater than that in the methanol mobile phase, while some of the humic acids were not eluted at all in either mobile phase, because humic acids have chemical as well as molecular diversity. Usually, humic acids can be easily adsorbed on relatively hydrophobic stationary phase such as C_{18} phase, probably through hydrophobic interaction, however, on the prepared polymer elution of humic acids was probably through size exclusion effect.

The elution of the majority of humic acids in the water mobile phase is strong evidence that 4-vinylpyridine, which was introduced by the molecular imprinting as the functional monomer, did not have an effective molecular interaction with the humic acids, because the pyridine functional group usually completely catches humic acids in aqueous mobile phase [17]. This is a surface selective localization effect of the functional group as well as the size exclusion effect of humic acids from the micropores of the adsorbents.

To examine concentration effect for bisphenol A, bisphenol A was concentrated from the water solution of bisphenol A with excess of humic acids. The result is shown in Fig. 3. The concentration magnification of bisphenol A was calculated as 115-fold in this case. Moreover, only bisphenol A was quantitatively concentrated, while humic acids were almost completely removed.

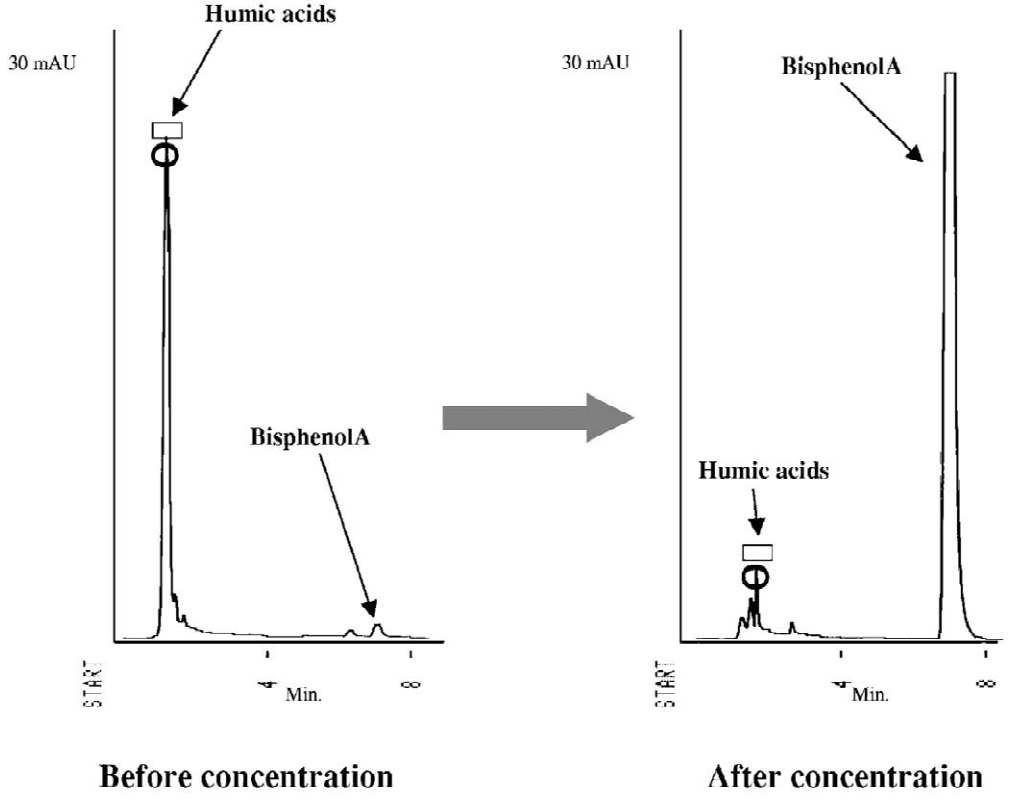


Fig. 3. Chromatograms of humic acids and bisphenol A before and after the treatment using the prepared polymer particle packed cartridge. HPLC conditions: mobile phase, 50% aqueous methanol; flow rate, 1.0 ml/min; detection, UV 254 nm; column, C_{18} column (Chromolith, Merck), 100 mm×4.6 mm I.D.; temperature, 30 °C.

If we applied 500 ng of bisphenol A on the polymer packed cartridge, over 95% of bisphenol A was recovered from the cartridge by simple elution using methanol solvent. If, instead of *p-tert.*-butylphenol as the template molecule, we utilized bisphenol A as the template molecule, up to 500–300% of bisphenol A was recovered. It is clearly strange, but as mentioned in the Introduction, bisphenol A utilized as the template molecule was also eluted during the adsorption and elution processes of bisphenol A even if the imprinted polymer was ''completely'' washed. Therefore, the traditional molecular imprinting method does not meet our purpose especially when we must treat dilute solution.

If we applied 100 ppb water solution of bisphenol A on the polymer packed cartridge, greater than 1000-fold quantitative concentration effect was obtained, while no serious contamination with humic acids occurred. This will be a great advantage for the analysis of quite low concentrations of bisphenol A.

In fact, when we treated environmental water with the polymer-packed cartridge, as shown in Fig. 4, the concentration of bisphenol A was effectively observed to afford a detectable peak, while the traditional C_{18} cartridge could not show the peak of bisphenol A with much larger solvent-front peaks. The size uniformity of the adsorbent is really important for the treatment of real environmental water, because various contaminants as well as dust make the viscosity of environmental water very high. This should prevent smooth flow of the environmental water through adsorbent cartridge. In our case described, very smooth flow was observed throughout the entire experiment. This is the great advantage of uniformly sized polymer adsorbents.

4. Conclusion

The molecularly imprinted polymer prepared using *p-tert.*-butylphenol as a pseudo template molecule effectively and quantitatively concentrated dilute bisphenol A up to 1000-fold, while almost complete removal of humic acids was observed. Since bisphenol A can not be utilized as the template molecule, this method can be a strong tool for analysis of bisphenol A in environmental water.

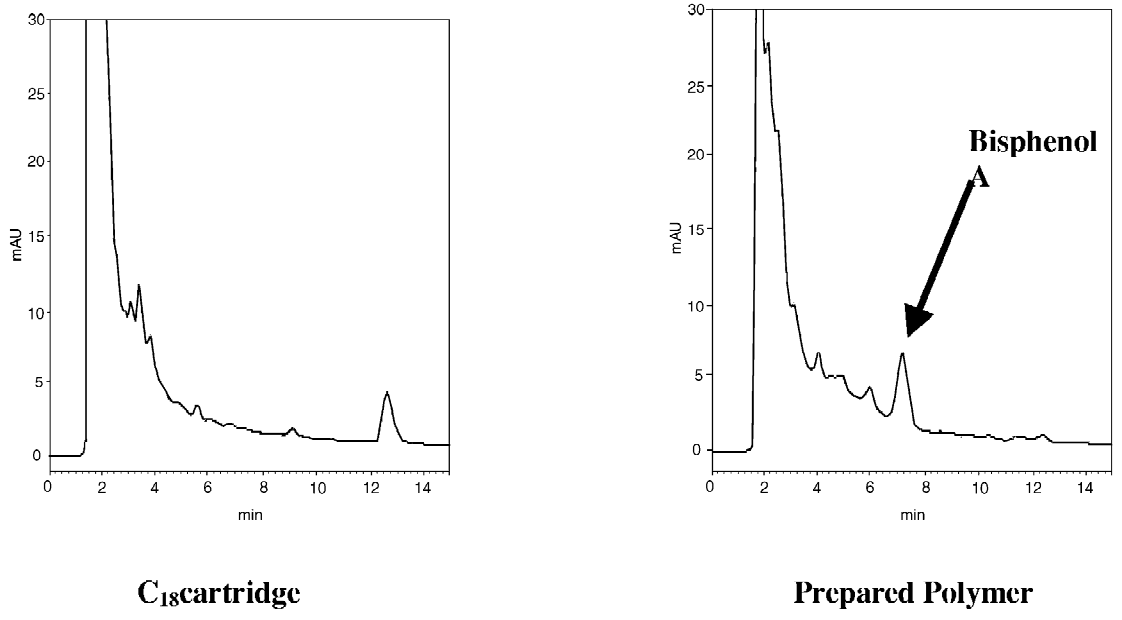


Fig. 4. Concentration effect on the prepared polymer particle packed cartridge for bisphenol A in environmental water. HPLC conditions: column, Mightysil (Kanto Chemicals); mobile phase, 50% aqueous MeOH; flow rate, 1.0 ml/min; detection; diode array detector; temperature, room temperature. Horizontal axis is in minutes, vertical axis is in mAU.

Acknowledgements

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