Novel Surface-modified Molecularly Imprinted Polymer Focused on the Removal of Interference in Environmental Water Samples

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(Received April 2, 2004; CL-040357)

Uniformly sized molecularly imprinted polymers (MIPs) for bisphenol A (BPA) with immobilized intervals of functional monomers utilizing 4,4′-methylenebisphenol as a pseudo template have been prepared. The MIPs through surface modification for the removal of interference showed significant selectivity for BPA retention and removal performance for interference in actual environmental water samples as the HPLC stationary phase compared to those of ordinary MIPs. These MIPs were employed as pretreatment media of column switching HPLC.

Molecularly imprinted polymers (MIPs) are widely used for the selective concentration and pretreatment of analytes in complex matrix such as drugs and their metabolites.[1] In environmental analysis, there are difficulties due to complex matrix, substantial interference, and very low concentrations of target chemicals contained in actual samples such as river and lake waters.[2] Bisphenol A (BPA) is frequently detected in environmental water and is attracting attention as an endocrine disrupter as it has rapidly entered the environment, food chains, and therefore the human diet. It has recently been reported that BPA shows estrogenic activity even at concentrations below 1 ppt,[3–5] therefore monitoring ultra low concentration of BPA in environmental water samples is important.

To overcome these difficulties, MIPs with highly specific binding capacity and the ability to remove interference is one solution.[6] Combined pretreatment with specific MIPs and chromatographic determination is the most promising procedure. We have developed newly designed MIPs for BPA pretreatment and applied them to the actual determination of BPA. In trace analysis, leakage of the residual template molecule, which is the same as the target molecule, prevents the accurate determination of the target compound.[7] Consequently a structurally related analog, which can be separated in the subsequent chromatographic process, is employed as an alternative template molecule. Previously *p-tert*-butylphenol (TBP) was used as a pseudo template,[8–10] but we used 4,4′-methylenebisphenol (MBP). As is shown in Figure 1, MBP is structurally closer to BPA than TBP. The uniformly sized MIP was prepared by a two-step swelling method.[11] During this process, 4-vinylpyridine (4VP) functional monomer, was introduced into ethylene glycol dimethacrylate (EDMA) as a cross-linking agent in the form of a complex with MBP template providing an effective interval of 4VP for BPA adsorption. Imprinted sites were then created by removing MBP after polymerization as shown in Figure 1.

Based on our preliminary investigations, the advantage of BPA retention on the MIP for free energy change on phase-transfer was calculated as 1.7 kJ/mol based on the comparison of retention factor (k′) of BPA and dipropyl phthalate, which has similar $\log P$ value to that of BPA. Excess 4VP was bonded onto EDMA in random intervals so there were two types of binding sites in the MIP. When the binding amount was small, highly selective imprinted sites were largely distributed in static conditions but in chromatographic retention these two types of binding sites acted simultaneously.[8,12] By Scatchard analysis, the association constant (Ka) of high affinity sites was 2.8×10^4 (M^{-1}) and for low affinity sites 1.2×10^3 (M^{-1}), which is almost the same value as for the non-imprinted polymer. The number of high affinity sites was smaller than that of low affinity sites but the MBP imprinted polymer showed a larger specificity for BPA retention than that of the TBP imprinted polymer. This was confirmed chromatographically by using these MIPs as HPLC stationary phases. Based on the results, the larger separation factor, the α (k′ of BPA/k′ of phenol) value of the MBP imprinted polymer was observed.

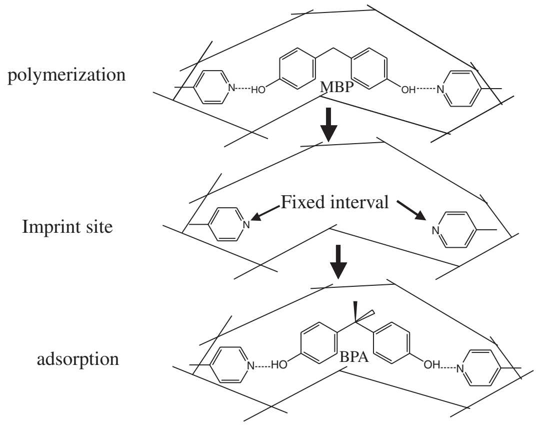

Figure 1. Simplified schematics of imprinting sites.

To remove interference in environmental water samples, a portion of the MBP imprinted polymer was surface-modified with methacrylic acid 3-sulfopropyl (MAS). Our preliminary investigation, revealed that the major interference in environmental water samples had a totally negative charge, so that repulsive force between the interference and the sulfopropyl group was expected to effectively remove of it. Even after surface modifications with MAS, the absolute retention factor of BPA on the MIP was larger than that of the non-surface modified MIP. This result means that molecular imprinting effect can be consistent with surface modification. Pretreatment columns packed with the MBP imprinted polymer and with its surface-modified polymer were used for column switching HPLC,[2] which provided highly reliable results for BPA determination when combined with electrochemical detection.[13–15] The detection limit for this method was 0.36 ppt.[16,17] The pump delivered 50 mL of sample water onto the pretreatment column packed with the MIP then the existing matrix were concentrated. The concentrated band containing BPA was led to the analytical column by changing the position of the flow change-over valve in the HPLC system.

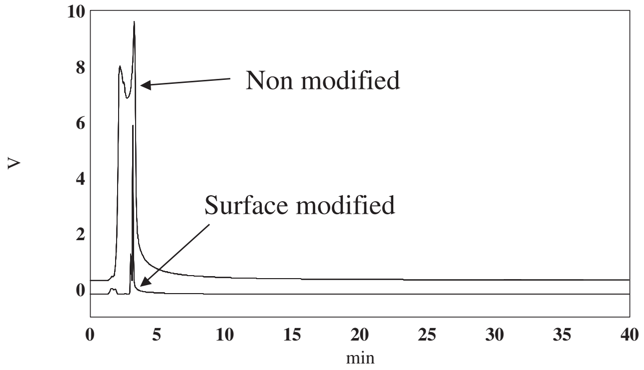

Figure 2. Comparative chromatograms of Suwannee River NOM obtained with/without surface modification of MIP used for the pretreatment column in the column switching HPLC system. HPLC conditions: mobile phase, 20 mM sodium phosphate buffer (pH 7.0)-acetonitrile (70:30 (v/v)); flow rate, 0.8 mL/min for analysis and 2.5 mL/min for pretreatment; column, Shim-pack VP-ODS (150 mmL × 4.6 mm i.d.); detection, UV 275 nm; temperature, 40 °C; concentration volume, 50 mL.

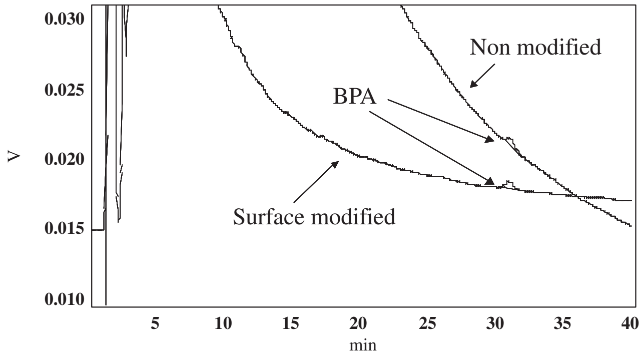

Figure 3. Close-up of Figure 2. The trace of "non-modified" is located at lower position than that in Figure 2 for ease of comparison.

We have reported that trace amounts of BPA in environmental water sample such as river and lake water can be determined with column switching HPLC coupled with electrochemical detection involving MIP as pretreatment media[14] but due to interference in actual samples, widely used UV detection could not be applied. In this study, we tried to determine the BPA concentration in actual environmental samples and Suwannee River NOM (natural organic matter), which is frequently used as a reference matrix in environmental analyses. We also tried to modify MIPs with a hydrophilic layer consisting of a 1:1 mixture of glycerol monomethacrylate and glycerol dimethacrylate. This modification was effective for electrochemical detection whereas it was not sufficient for UV detection. When the MAS-modified MIP was employed as the pretreatment medium of the column switching HPLC system, the BPA contained in Suwannee River NOM could be detected even with UV detection as is shown in Figures 2 and 3. For reference, the chromatogram obtained with the MBP imprinted non-surface modified MIP is shown in the same figures.

Surface modification with MAS provided highly reliable results. The BPA concentration detected in the NOM sample solution was around 200 ppt and the sample solution was prepared by adding 5 mg of NOM into 200 mL of pure water and then mixing. Using this sample preparation, the BPA content in the NOM was approximately 10 ng/mg.

In conclusion, a molecularly imprinted polymer was successfully applied to BPA determination in environmental samples such as river water and Suwannee River NOM.

MBP as a pseudo template provided improved selectivity to the MIP based on its specific imprint site and immobilized interval of functional monomers. Surface modification of the MIP with MAS afforded a significant effect for reducing the interference in the resultant chromatogram and according to this effect, BPA contained in Suwannee River NOM was determined using UV detection.

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Published on the web (Advance View) June 7, 2004; DOI 10.1246/cl.2004.806