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Novel surface modified molecularly imprinted polymer focused on the removal of interference in environmental water samples for chromatographic determination

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Abstract

Uniformly sized molecularly imprinted polymers (MIPs) for bisphenol A (BPA) with surface modification and immobilized intervals of functional monomers afforded by utilizing 4,4′-methylenebisphenol as a pseudo component have been prepared. MIPs for BPA were prepared using 4-vinyl pyridine immobilized in the most effective interval and ethylene glycol dimethacrylate as a functional monomer and cross-linking agent, respectively. Prepared MIPs showed significant selectivity for BPA retention and removal performance for interference in actual samples as the HPLC stationary phase compared to those of ordinary MIPs. These MIPs were employed as pretreatment media of column switching HPLC and the HPLC system provided a detection limit of 0.36 ppt when electrochemical detection was used. Actual samples, including Suwannee River natural organic matter (NOM), were applied and BPA was detected in the NOM even if widely used UV detection was employed.

Keywords: Bisphenol A; Molecularly imprinted polymers; Surface modification; Column switching HPLC

1. Introduction

Molecularly imprinted polymers (MIPs) are widely used for the selective concentration and pretreatment of target compounds existing in complex matrix such as plasma [1]. In environmental analysis, very low concentration of target chemicals contained in actual samples such as river and lake waters occurs serious difficulties in the sample preparation due to substantial interference [2].

Bisphenol A (BPA) is frequently detected in environmental water and is attracting attention as an endocrine disrupter because 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 ng/l (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 of the solutions [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 [8,9], however, we used 4,4′-methylenebisphenol

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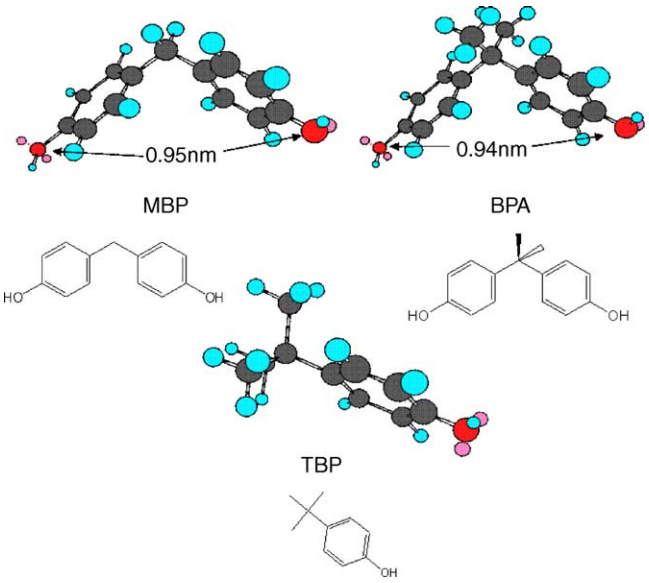

Fig. 1. Three-dimensional structures of pseudo-templates and BPA.

(MBP). As is shown in Fig. 1, MBP is structurally closer to BPA than TBP. The uniformly sized MIP was prepared by a two-step swelling method [10]. 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, which interacts with hydroxyl group of BPA. Imprinted sites were then created by removing MBP after polymerization. Created appropriate interval of 4VP afforded increase of interaction with BPA due to the effective hydrogen bonding with 4VP and hydroxyl group of BPA. Figs. 2 and 3 show simplified schematics of imprinted sites created with TBP and MBP, respectively.

To remove interference in environmental water samples, a portion of the MIPs was surface modified with methacrylic acid 3-sulfopropyl (MAS) and other hydrophilic monomers of glycerol dimethacrylate (GDMA) and/or glycerol monomethacrylate (GMMA).

Obtained surface modified MIPs were evaluated through HPLC, nitrogen adsorption method and Schatchard analyses. Pretreatment columns packed with the MBP imprinted polymer and with its surface modified polymers were used for column switching HPLC [2], which provided highly reliable results for BPA determination when combined with electrochemical detection [11–13]. The detection limit for this method was 0.36 ppt [14,15].

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 [15]. But due to interference in actual samples, widely used UV detection could not be applied in general. In this study, we tried to confirm the effect of surface modification onto MIPs by applying them to actual HPLC analysis of BPA in environmental samples such as Suwannee River natural organic matter (NOM), which was collected from the same site that was used originally to collect the standard Suwannee River humic and fulvic acids and frequently used as a reference matrix in environmental analysis.

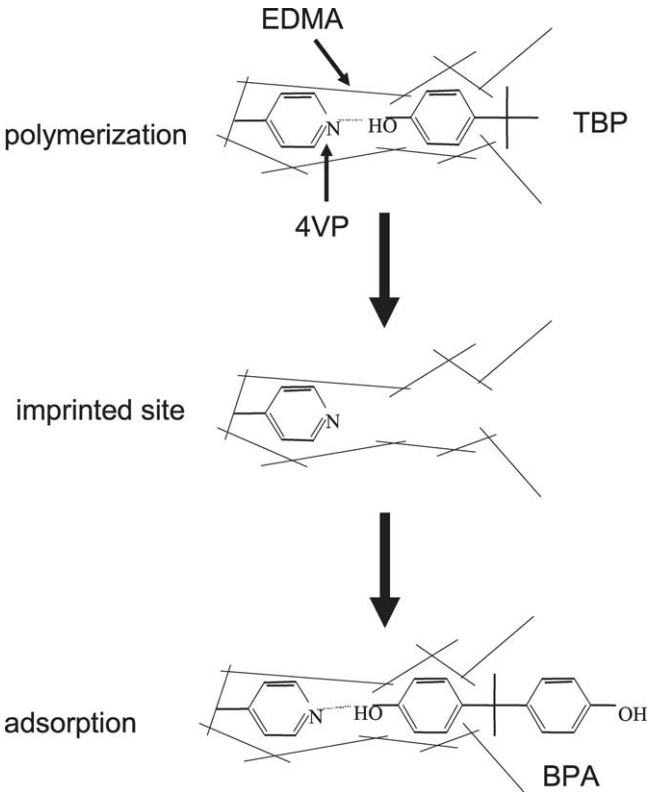

Fig. 2. Simplified schematics of creating TBP imprinted procedures.

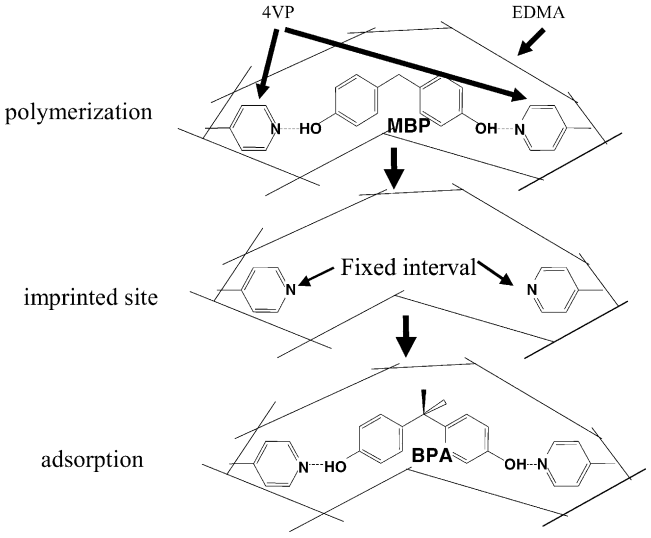

Fig. 3. Simplified schematics of MBP imprinted sites.

2. Experimental

2.1. Materials

Monomers, ethylene glycol dimethacrylate (EDMA) as a cross-linking agent, and 4-vinylpyridine (4VP) as the functional monomer, both from Wako Pure Chemicals (Osaka, Japan) were effectively purified by vacuum distillation techniques to remove polymerization inhibitor [16]. Glycerol dimethacrylate (GDMA) and glycerol monomethacrylate (GMMA) were purchased from Kyoeisya Chemical (Osaka, Japan) and used without further purification. The template molecule, *p-tert*-butyphenol (TBP) was purchased from Nacalai Tesque (Kyoto, Japan) and 4,4′-methylenebisphenol (MBP) and butyl methacrylate (BMA) were purchased from Wako Pure Chemicals. A polymerization initiator, 2,2′-azobis-(2,4-dimethyvaleronitrile) (ADVN) and benzoyl peroxide were purchased from Wako Pure Chemicals. Bisphenol A (BPA) and a solvent realizing porous structure (porogenic solvent), toluene from Nacalai Tesque was of the highest grade. Suwannee River NOM was purchased from International Humic Substances Society (St. Paul, MN, USA).

All chemicals for preparing HPLC mobile phase, sodium dihydrogen phosphate, disodium hydrogenphosphate and acetonitrile were purchased from Wako Pure Chemicals. Water for preparing BPA standard solution was obtained from Milli-Q water purification system of Millipore (Bedford, MA, USA), and furthermore purified with empore disk (47 mm o.d. of SDB-XD type) from 3M (St. Paul, MN, USA).

2.2. Preparation of the molecularly imprinted polymer

Uniformly sized polystyrene seed particles were prepared by a typical emulsifier-free emulsion polymerization method and purified by a centrifugation method. The size of seed particles was around 1 μm in diameter with excellent size mono disperse.

Preparation of uniformly sized macro-porous polymer particle by a multi-step swelling and polymerization method [17] was carried out as follows. In the first step, 0.162 ml of aqueous dispersion of the purified polystyrene seed particles (2.23×10^{-1} ml/ml) was admixed with micro-emulsion of 0.167 ml of dibutyl phthalate (activating solvent), 0.04 g of sodium dodecylsulfate, and 10 ml of distilled water by sonication.

This first step swelling was carried out at room temperature while the solution was stirred at 125 rpm. Completion of the first step swelling was determined by the vanishing point of oil droplets in added micro-emulsion using an optical microscope.

A dispersion of 3 ml of toluene (porogenic solvent), 0.34 ml of 4VP, 0.06 g of TBP or 0.08 g of MBP, 0.15 g of ADVN and 0.06 g of sodium dodecylsulfate into 35 ml of water containing 0.45 g of poly(vinyl alcohol) (degree of polymerization, DP = 2000; saponification value = 86.5–89 mol%) as dispersion stabilizer was added to the dispersion of swollen seed particles. This second step swelling was carried out at room temperature with stirring at 125 rpm.

After the second step swelling was completed, the other dispersion of 3 ml of EDMA, 0.06 g of sodium dodecylsulfate into 35 ml of water containing 0.45 g of poly(vinyl alcohol) was added to the dispersion of the swollen particles. This swelling step was carried out for 6 h at room temperature while the solution was stirred at 125 rpm. For the polymerization of swollen particles, the aqueous dispersion was stirred at 50 °C for 24 h under argon atmosphere. The polymer particles obtained were washed with water, methanol, and tetrahydrofuran to remove the porogenic solvent, template molecules other impurities. The feed ratio was as follows, EDMA 4VP-template, 40:8:1 in mole ratio.

Some of obtained MIPs were surface modified as described in following sections.

2.3. Surface modification methods

0.8 g of MIPs (base polymer particles) prepared using the multi-step swelling and polymerization method were dispersed in 50 ml of acetone and the hydrophilic monomers of a mixture of GMMA and GDMA (0.3 g), same as described previous section and ADVN were added (5% in weight ration of monomers) and polymerized at refluxing temperature of acetone. The polymerization was continued for 24 h and the obtained particles were washed with acetone and water in order.

The base polymer particles were dispersed in 50 ml of methanol and the ionic monomer of methacrylic acid 3-sulfopropyl (MAS) potassium salt (0.3 g) and benzoyl peroxide (BPO) were added (5% in weight ratio to monomer) and polymerized at refluxing temperature of methanol. The polymerization was continued for 24 h and the obtained particles were washed with methanol and 1N HCl and water in order.

2.4. Column packing method

The prepared particles were packed into stainless steel columns (30 mm × 4.6 mm) by slurry techniques to evaluate their characteristics. We mainly utilized mixture of water, isopropanol, and methanol as packing medium.

2.5. Chromatographic measurement

HPLC measurement was carried out with the LC–VP HPLC system from Shimadzu (Kyoto, Japan) consisted of a LC-10Avp solvent delivery pump, CTO-10Avp column oven, FCV-12AH two-position flow changeover valve, FCV-13AL six-port flow selection valve, SIL-10Avp automatic injector, Rheodyne 7725 manual injector (Cotati, CA, USA) with 100 μl loop, SCL-10A system controller and a CLASS-VP work station software. A Coulochem II, electrochemical detector (ECD) was purchased from ESA (Chelmsford, MA, USA).

Small hydrocarbons including BPA were analyzed by HPLC to compare the retention times on respective MIPs.

HPLC conditions for small hydrocarbons were as follows: mobile phase, water–acetonitrile, 55/45 (v/v); flow rate, 0.3 ml/min; detection, UV 220 nm; temperature, 40 °C; column, packed with prepared MIPs (30 mm × 4.6 mm).

2.6. Pore size measurement

Pore size, pore size distribution and specific surface area measurements were carried out with a Micromeritics Gemini 2375 along with a Micromeritics Flow Prep 060 using a standard BET calculation.

2.7. Scatchard analysis

10 mg of EDMA (NIP, reference particles), TBP imprinted, TBA imprinted-MAS modified and TBP imprinted-butyl methacrylate modified, a reference as hydrophobic modification, were added to a 2 ml of acetonitrile solution of BPA of known concentrations (2×10^{-3} to 2×10^{-1} mM) in vials, respectively. Each concentration was prepared in duplicate. After the resulting suspension was rotated for 15 h at 25 °C, a fraction (1 ml) of each BPA-incubated sample was taken and filtrated with 0.45 μm of membrane filter. The concentration of free BPA, [BPA], was determined by HPLC with VP-ODS column (150 mm × 4.6 mm, Shimadzu) at UV 220 nm. [BPA] was determined as an average value of two measurements. The amount of BPA bound to MIPs, n, was calculated by subtraction of [BPA] from the initial BPA concentration. Scatchard analysis was provided by the Scatchard equation, $n/[\mathrm{BPA}] = (N - n)K_a$, where K_a is an association constant and N is an apparent maximum number of binding sites. Therefore, K_a and N could be determined from the slope and the intercept, respectively, by plotting of $n/[\mathrm{BPA}]$ versus n.

2.8. Chromatographic applications

Surface modified MIPs were adopted as pretreatment columns of the column switching HPLC and applied to actual determination of BPA. To accomplish suppression of BPA contamination and determine the BPA concentration in water samples, a special technique was required. A column switching HPLC with a pump injection system was one of the solutions. Fig. 4 shows flow diagram of the column switching HPLC system used in this study. The pump delivered 50 ml of BPA standard solutions or environmental water samples and the BPA was concentrated on the pretreatment column. Then mobile phase was delivered via a six-port switching valve and then the concentrated BPA was directed to the analytical column and detected by the detector after the separation on the analytical column.

HPLC conditions employed for column switching HPLC were as follows: mobile phase, 20 mM (sodium) phosphate buffer (pH 7)/acetonitrile, 65/35 (v/v); rinsing solvent, 20% (v/v) of acetonitrile aqueous solution; flow rate for analysis, 0.8 ml/min; flow rate for pre-treatment, 2.5 ml/min; concentrated volume, 50 ml; analytical column, Shim-pack VP-ODS (150 mm × 4.6 mm); temperature, 40 °C; electrochemical detection, at +0.35/+0.55 V (analytical cell, CH1/CH2, 1 μA F.S.) and +0.6 V (guard cell); UV detection, at 220 or 275 nm.

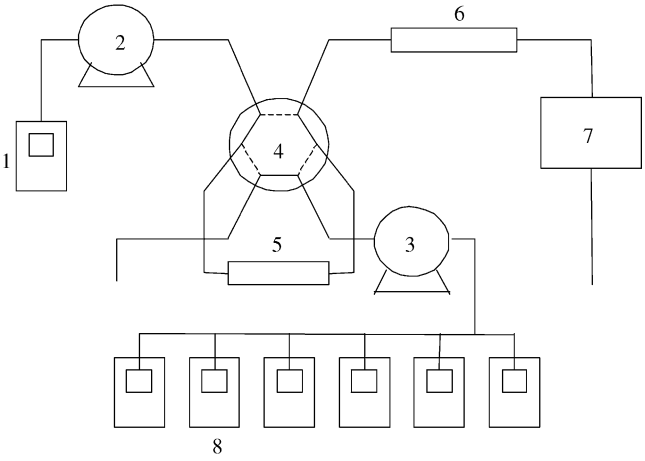

Fig. 4. Flow diagram of column switching HPLC for auto-pretreatment. (1) Mobile phase; (2 and 3) pump; (4) high pressure flow changeover valve; (5) pretreatment column; (6) analytical column; (7) detector; (8) sample (one for rinsing solvent).

For the reference, LCMS analysis was compare to HPLC column switching analysis.

3. Results and discussion

3.1. Effect of newly employed template MBP

Prepared MIPs are shown in Table 1 (MIP-TN, MIP-TG, MIP-TM, MIP-TB, MIP-MN, MIP-MM, and NIP).

The particle size of those MIPs is 8 μm in diameter. The size uniformity of the polymer particles was excellent as reported previously [17]. The observation of those particles by scanning microscopy is shown in Fig. 5.

We estimated the advantage of MIP in chromatographic retention preliminarily along following procedures. NIP and MIP-TN were packed into stainless steel column (30 mm × 4 mm) then BPA and dipropylphthalate, which has

Table 1
Specifications of prepared MIPs

Name of MIP	Template	Surface modifier
NIP	None	None
MIP-TN	TBP	None
MIP-TG	TBP	GDMA/GMMA
MIP-TM	TBP	MAS
MIP-TB	TBP	BMA
MIP-MN	MBP	None
MIP-MM	MBP	MAS

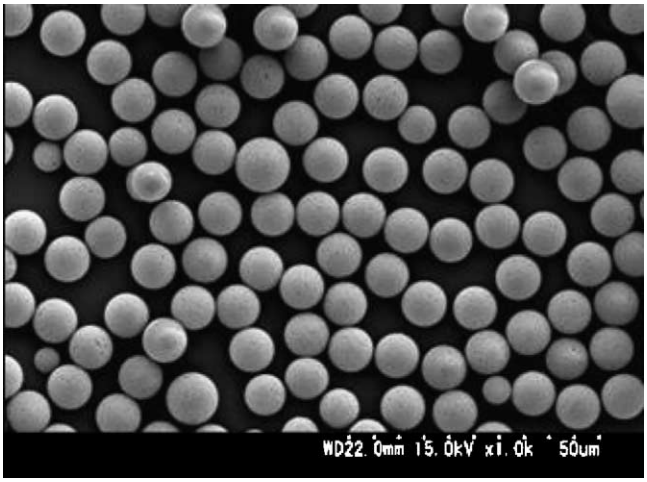

Fig. 5. Photograph of MIP-TG particles obtained by scanning microscopy. Magnification: 1000×.

similar log *P* value to that of BPA were injected under the same conditions described in Fig. 9. The advantage of BPA retention on the TBP based MIP for free energy change on phase-transfer was calculated as 1.7 kJ/mol based on the comparison of retention factors (k) of BPA and dipropylphthalate by using the equation of $\Delta G = RT \ln k$, where phase ratio was presumed as 1. The comparison of the effects afforded by TBP and MBP as template molecules is shown in Fig. 6. Retention factor k was calculated preliminary from the equation $k = (t_R - t_0)/t_0$, where t_R and t_0 are retention times of retained and non-retained solutes obtained by HPLC analyses, respectively. The BPA separation factor was calculated from the equation $\alpha_{BPA/phenol} = k_{BPA}/k_{phenol}$, where k_{BPA} and k_{phenol} are the retention factors of BPA and phenol, which is one of related analogues of BPA, on respective MIPs.

Some of 4VP was introduced into imprinted sites in the form of a complex with MBP providing an effective interval of 4VP for BPA adsorption. MBP-imprinted MIP afforded larger selectivity for BPA adsorption against phenol than that of TBP-imprinted MIP as is shown in Fig. 6. Effective interval of 4VP immobilized in imprinted site and higher similarity

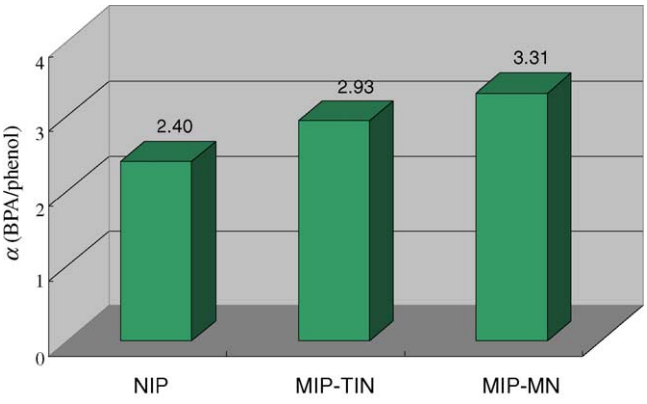

Fig. 6. Comparison of retention selectivity for BPA retention on MIPs caused by different templates.

Table 2
Comparison of MIPs with/without surface modification

	BET		
	Specific surface area (m^2/g)	Total pore volume (cm^3/g)	Mean diameter (nm)
MIP-TG	110.5	0.237	9.42
MIP-TN	417.9	0.621	5.94

of imprinted site to target BPA than that afforded by TBP-template showed proper hydrogen bonding and hydrophobic interaction.

3.2. Evaluation of surface modification

Surface modification was carried out to remove interference in environmental water samples. The interference may be considered water-soluble oligomer such as humic materials. Consequently, pore volume and/or pore size control with hydrophilic polymer coating is easily expected to be effective.

The results of pore size measurement for MIPs with BET are shown in Table 2 and Fig. 7. The total pore volume, specific surface area and mean diameter of surface modified MIP (MIP-TG, GDMA/GMMA as modifier) obtained by statistical method are different from those of non-modified MIP (MIP-TN) and Fig. 7 shows the difference of pore-distribution in meso-pore range (1–10 nm as a diameter). In this surface modification method, monomers for surface modification are dissolved into solvent medium such as acetone or methanol then the inside of the meso-pore may be filled with the solvent containing the modifier because monomer size is almost within the range of meso-pore size and then polymerized. Hence, it is suggested that large number of meso-pores are modified with the modifier monomer in dispersion method. A schematic image of the modification is shown in Fig. 8.

Hydrophilic surface modification with GDMA/GMMA or MSA decreases hydrophobic retention capacity in general. But Fig. 9 shows that absolute retention capacity of BPA on MAS modified MIP (MIP-MM) was larger than that on non-imprinted polymer (NIP), whereas other small hydrocarbons showed significantly smaller retentions on MIP-MM. This fact may be explained by the effect of MBP template, which provided highly selective adsorption sites due to fixed interval of functional monomers (4VP). The advantage of large retention can be kept despite the hydrophilic surface modification.

3.3. Scatchard analysis

If Langmuir models can be applied, the association constant K_a and specific site capacity N can be determined from the slope and y intercept of lines obtained by least-squares regression of linear regions of the corresponding Scatchard plots.

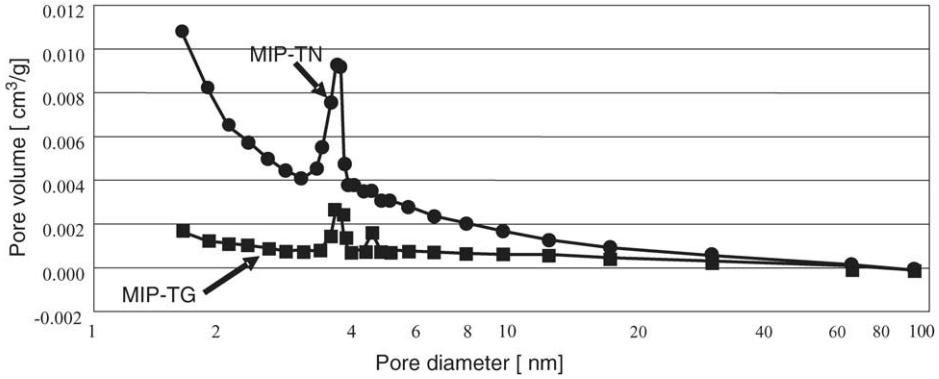

Fig. 7. Distribution of pore diameter for MIP-TN and MIP-TG measured by nitrogen adsorption method.

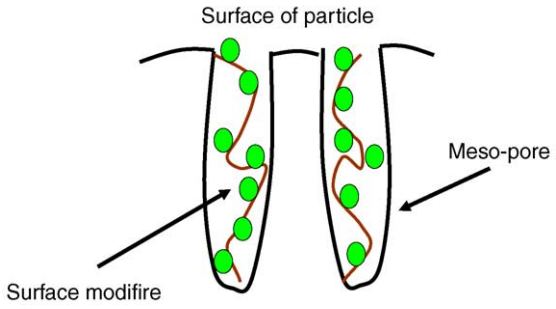

Fig. 8. Schematic image of the surface modified polymer.

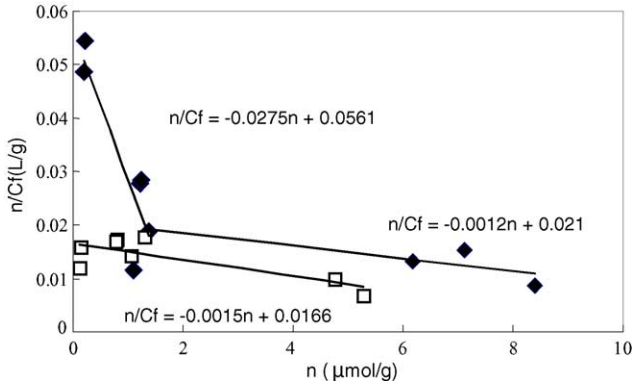

Fig. 10. Scatchard plots of MIP and NIP. TBP imprinted MIP (MIP-TN) (♦); non-imprinted blank (NIP) (□). These plots were obtained based on following equation: $n/[BPA] = (N - n)K_a$, where K_a is an association constant and N is an apparent maximum number of binding sites. [BPA] was measured by HPLC under following conditions: mobile phase, water–acetonitrile, 45/55 (v/v); flow rate, 0.8 ml/min; detection, UV 220 nm; temperature, 40 °C; column: Shim-pack VP-ODS (150 mm × 4.6 mm).

Scatchard plots of MIPs were non-linear as shown in Fig. 10. Two straight lines can be drawn, indicating that the affinities of the binding sites in MIPs are heterogeneous and can be approximated by two dissociation-constants corresponding to the high- and low-affinity binding sites.

The above phenomena in the Scatchard analysis are usual for MIPs [18–20]. Fig. 10 shows a comparison of NIP, blank polymer (non-imprinted), and MIP-TN, TBP imprinted and no-surface modified.

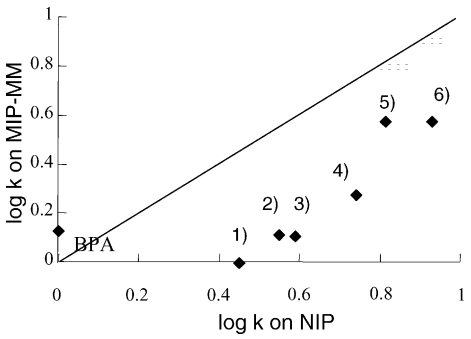

Fig. 9. log k–log k plot of MAS modified MIP vs. non-imprinted polymer. Points identification are: (1) butyl benzene; (2) amyl benzene; (3) triptycene; (4) *o*-terphenyl; (5) pyrene; and (6) triphenylene from left to right. HPLC conditions were as follows: columns, packed with MIP and non-imprinted polymer, respectively (30 mm × 4 mm); mobile phase, water–acetonitrile, 40:60 (v/v); flow rate, 0.3 ml/min; and temperature, 40 °C.

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 contributed largely in static conditions but in chromatographic retention these two types of binding sites acted simultaneously [21,22].

Table 3
Association constants and specific binding capacities of MIPs

	K_a (M^{-1})	N (μmol/g)
MIP-TM (high affinity)	2.7×10^4	1.2
MIP-TM (low affinity)	2.1×10^3	5.7
MIP-TB (high affinity)	3.1×10^4	3.0
MIP-TB (low affinity)	6.0×10^2	33
MIP-TN (high affinity)	2.8×10^4	2.0
MIP-TN (low affinity)	1.2×10^3	17.5
NIP	1.5×10^3	11.1

HPLC conditions for these measurements were as follows: mobile phase, water–acetonitrile, 45/55 (v/v); flow rate, 0.8 ml/min; detection, UV 220 nm; temperature, 40 °C; column, Shim-pack VP-ODS (150 mm × 4.6mm); injection volume, 5 μl. BPA concentration in NOM was approximately 10 ng/g.

Similar Scatchard plots were obtained for MIP-TM and MIP-TB. MIP-TM was obtained through surface modification of MIP-TN with MAS, hydrophilic coating where as MIP-TB was obtained with BMA, hydrophobic coating.

The properties of binding sites in MIPs are summarized in Table 3. For MIP-TN, the association constant (K_a) of high affinity sites was $2.8 \times 10^4\ M^{-1}$ and for low affinity sites $1.2 \times 10^3\ M^{-1}$, which is almost same value as for the non-imprinted polymer. The number of high affinity sites was smaller than that of low affinity sites but MIP-TN showed a larger specificity for BPA retention than that of NIP. This was confirmed chromatographically by using these MIPs as HPLC stationary phases.

It is interesting to note that K_a and N values of high affinity sites of MIP-TN, MIP-TM and MIP-TB are rather similar. On the other hand, those of low affinity sites of MIP-TN, MIP-TM and MIP-TB are largely different. In the case of MIP-TM, N is smaller than that of MIP-TN and chromatographic analysis exhibited affirmative results of small retention factors of BPA and other hydrophobic analytes. In the case of MIP-TB, N is larger and retention factors of BPA and other hydrophobic analytes was large. These facts suggest that surface modification to MIPs affect mainly low affinity sites whereas high affinity sites maintain large K_a value even after hydrophobic and hydrophilic surface modifications.

3.4. Chromatographic applications

In our preliminary investigation, the complex interference appeared in the fore part of chromatograms was isolated by preparative HPLC then re-injected onto both anion and cation exchange columns under appropriate conditions. The interference was retained on the anion exchange column whereas not retained on the cation exchange column. That means the major interference in environmental water samples was negatively charged totally, so that repulsive force between the interference and the sulfopropyl group of MAS was expected to remove of it effectively.

Fig. 11 shows a comparison of the removal capacity of TBP imprinted MIPs based on the difference of surface modification when actual water sample was applied. MIP-TM, MAS modified MIP, showed significant effect for the removal of interference. MIP-TG showed superior removal of interference to that of MIP-TN but not so effective as of MIP-TM. MIP-TB, modified with hydrophobic BMA, showed larger interference peak than that of MIP-TN when different water sample was applied under same HPLC conditions as described in Fig. 11. Due to the difference of actual sample, the chromatogram was not shown in Fig. 11.

Even with MAS-modifications, which decrease hydrophobicity of MIPs, MIP-MM had more selective MBP-imprinted sites than those created with TBP. The absolute retention factor of BPA on MIP-MM was larger than that on MIP-MN, which was non-surface modified MIP with MBP template.

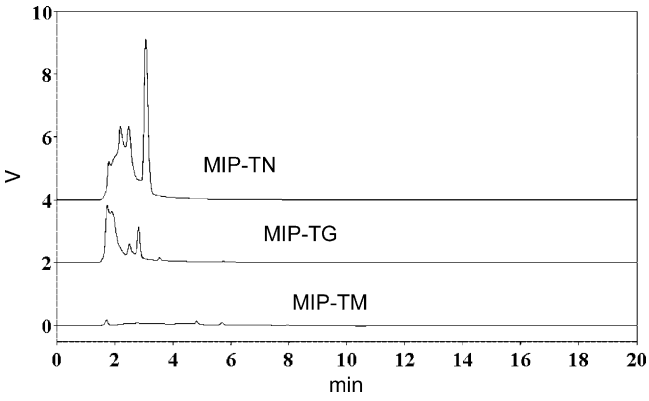

Fig. 11. Comparative chromatograms of lake water obtained MAS- (MIP-TM), GDMA/GMMA- (MIP-TG), non- (MIP-TN) modified MIPs used for the pretreatment column in the column switching HPLC system. HPLC conditions: mobile phase, 20 mM sodium phosphate buffer (pH 7.0)–acetonitrile (70:30 (v/v)); flow rate, 0.8 ml/min for analysis and 2.5 ml/min for pretreatment; column, Shim-pack VP-ODS (150 mm × 4.6 mm); detection, UV 220 nm; temperature, 40 °C; concentration volume, 50 ml.

This result means molecular imprinting effect can be consistent with surface modification. MIP-MN and MIP-MM were applied to column switching HPLC. The pump delivered 50 ml of sample water onto the pretreatment column packed with the MIP then the existing matrixes were concentrated. The concentrated band containing BPA was directed to the analytical column by changing the position of the flow change-over valve in the HPLC system.

When the MAS modified MIP (MIP-MM) was employed as a pretreatment medium of the column switching HPLC system, BPA contained in Suwannee River NOM could be detected even with UV detection as is shown in Figs. 12 and 13. For reference, a chromatogram obtained with the MBP imprinted non-surface modified MIP (MIP-TM) is shown in the same figures. The corresponding chromatogram obtained with electrochemical detection is shown in Fig. 14.

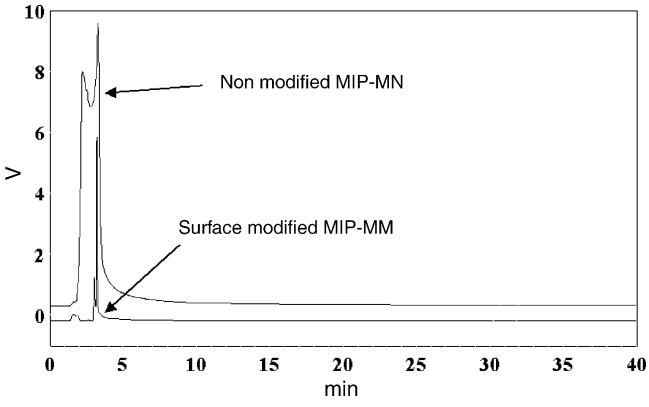

Fig. 12. Comparative chromatograms of Suwannee River NOM obtained with (MIP-MM)/without (MIP-MN) surface modification of MIP used for the pretreatment column in the column switching HPLC system. HPLC conditions: mobile phase, 20 mM sodium phosphate buffer (pH 7.0)–acetonitrile (70:30 (v/v)); flow rate, 0.8 ml/min for analysis and 2.5 ml/min for pretreatment; column, Shim-pack VP-ODS (150 mm × 4.6 mm); detection, UV 275 nm; temperature, 40 °C; concentration volume, 50 ml.

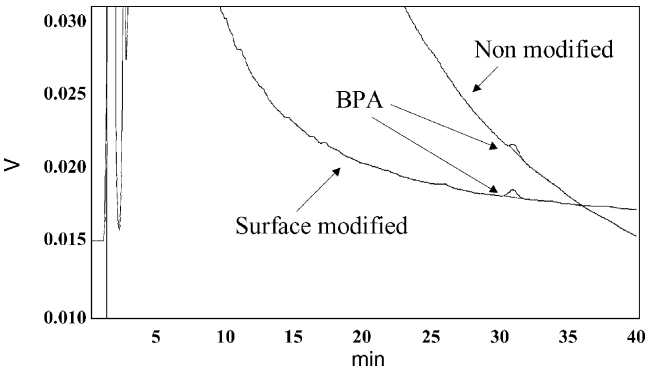

Fig. 13. Close-up of Fig. 12. The trace of "non-modified" is located at lower position than that in Fig. 12 for ease of comparison.

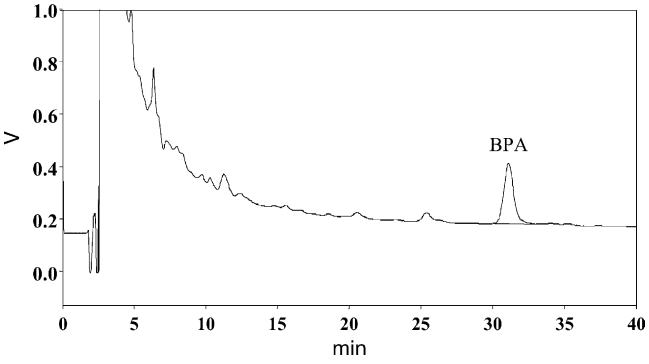

Fig. 14. Chromatogram of the same NOM sample as in Figs. 12 and 13 with electrochemical detection. HPLC conditions were same as those of Fig. 4 except for electrode voltage at 0.55 V.

BPA concentration in NOM was approximately 10 ng/g.

Surface modification with MAS provided highly reliable results.

Ordinary LCMS detection with TIC and SIM was carried out on 80-time concentrated NOM solution (50 μl injection) for column switching HPLC-UV detection then no corresponding peak was appeared in the chromatograms. This means selective LCMS detection cannot be applied to trace determination of BPA in complicated matrix as it is.

4. Conclusion

This work shows two innovations, one is that newly designed uniformly sized MIPs for BPA adsorption prepared with MBP as a pseudo-template was more effective than ordinary TBP-imprinted MIPs for their chromatographic retention and selectivity. The other is that surface modification with MAS for polymer based adsorbent including MIPs is a potential weapon for their application to practical field involving pretreatment for environmental analysis.

MAS-modified MIP was applied to actual pretreatment of BPA analysis with column switching HPLC and successful results of effective removal of interference were confirmed.

As a fundamental investigation, Scatchard analysis gave information in respect to the mechanism of adsorption on surface modified MIPs having two types of adsorption sites, high affinity sites and low affinity sites. In other words, the former is selective sites and the latter is non-selective sites. Through the surface modification by both hydrophilic and hydrophobic monomers, the highly selective sites in MIPs were maintained. This fact indicates that surface modification for MIPs can be designed independently from imprinting designation.

Surface modification technique for MIPs may influence their dissemination to applicable fields such as food processing, drug discovery, and biological analysis besides environmental applications and should be expected to be used column switching LCMS.

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