Molecularly Imprinted Microspheres for Bisphenol A Prepared Using a Microfluidic Device

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Spherical molecularly imprinted polymer particles for bisphenol A (BPA-MIP) were easily prepared by using a Y-junction microfluidic device. The sizes of the obtained BPA-MIP particles were found to be 86 μm with a narrow size distribution. The binding characteristics were investigated by drawing a binding isotherm to estimate the binding constant and by switching the polarity of solvents to examine the feasibility of use as a medium for affinity chromatography. When dichloromethane was used as a solvent, BPA was strongly bound to the spherical BPA-MIP particles based on hydrogen bond formation; after switching the solvent to methanol, BPA was eluted quantitatively due to the weakening of the hydrogen bonding, suggesting that the spherical BPA-MIP particles can be applied to affinity-type solid-phase extraction for BPA. As the present method can provide a diverse range of spherical MIPs without tedious procedures, MIP-based affinity media will be able to be more readily used as pretreatment and/or purification for various fields.

(Received January 30, 2012; Accepted March 19, 2012; Published May 10, 2012)

Introduction

Solid-phase extraction (SPE) is a sample pretreatment method for the concentration of analytes using solid-phase adsorbents. In the method, analytes are adsorbed on the adsorbent, depending on their chemical properties such as hydrophobicity, electrostatic force and so on, followed by washing to remove impurities and recovering the target analytes. The method is widely used as a pretreatment in bio-analyses and environmental analyses. Several types of stationary phases are commercially available, which are based on silica gels and polymers having functional groups interacting with target molecules hydrophobically and/or electrostatically.1-3 Affinity-based SPE has gained a great reputation; especially, molecularly imprinted polymers (MIPs) have often been used for this purpose, as they can be prepared easily without the use of natural antibodies and have given specific binding towards target molecules.4,5 Molecular imprinting is one of template polymerization techniques. It is conducted by co-polymerization of complexes, formed from a target or its derivative (template molecule) and a functional monomer, with a cross-linking agent.6-10 Binding cavities complementary to the template molecule in size and shape are left in the resulting polymer after the template molecule is removed. Traditional MIPs have been prepared by bulk polymerization, subsequently ground and sieved. The obtained polymers have irregular shapes, and often result in great losses of materials. When one considers applications such as solid-phase extraction and liquid chromatography, one can conclude that it is better to make polymers uniform and spherical. Because theoretical plate numbers tend to be higher, resolution of peaks becomes better when the spherical and monodispersed polymer particles are used as stationary phases.

Suspension polymerization is a simple and easy method to obtain a microsphere. For chromatographic stationary phase, MIPs prepared by suspension polymerization were reported previously.11-15 In this method, size-dispersion of polymer particles could be easily affected by experimental conditions such as the stirring rate; thus, it is difficult to control the size of droplets so as to obtain polymer particles bearing a narrow size distribution. A microfluidic device is reported to be a tool for producing uniform droplets.16-20 Such a device enables us to control not only the diameter but also the morphology of the obtained polymer particles by design of flow channels and mixing ratios of organic and aqueous phases and flow rates.

In this study, we prepared spherical imprinted polymer particles by using a Y-junction microfluidic device.21 Bisphenol A (BPA) was selected as a model target compound, as it is believed that BPA may act as an estrogen-like endocrine disruptor,22 and still needs to be monitored. BPA-imprinted polymers have often been prepared with BPA dimethacrylate or diacrylate as a template molecule.23-25 Recently, BPA di(4-vinylbenzoate) was found to give highly selective BPA-imprinted polymers;26 thus, this template was adopted for this study. The pre-polymerization solution and poly(vinyl alcohol) solution were introduced into the Y-junction microfluidic device as an organic phase and an aqueous phase, respectively, and spherical droplets were obtained from the exit of the microfluidic device. Following heating to initialize the polymerization, monodisperse polymer particles were prepared, and after the removal of the BPA moiety, the BPA-imprinted spherical polymer (BPA-MIP) particles were obtained. Further, a solid-phase extraction was demonstrated by using the obtained BPA-MIP as an affinity-type chromatography medium.

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**Experimental**

**Materials**

4,4'-Dihydroxy-2,2-diphenylpropane (bisphenol A, BPA), styrene, dichloromethane (CH$_2$Cl$_2$), sodium hydroxide (NaOH), acetonitrile (CH$_3$CN), 4,4'-azobis(2,4-dimethylvaleronitrile) (V-65) were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) was purchased from Peptide Institute, Inc. (Osaka, Japan). Poly(vinyl alcohol) (PVA, degree of polymerization 500) was purchased from Katayama Chemical Industries Co., Ltd. (Osaka, Japan). Styrene, DVB, magnesium sulfide, methanol (MeOH), and 2,2'-azobis(2,4-dimethylvaleronitrile) (V-65) were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Hexestrol, divinylbenzene (DVB), sodium hydroxide (NaOH), 4-dimethylaminopyridine (4-DMAP) were purchased from Nacalai Tesque (Kyoto, Japan). Bisphenol B (BPB) and 4-vinylbenzoic acid (4-VBA) were purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Heestrol, divinylbenzene (DVB), magnesium sulfate, methanol (MeOH), and 2,2'-azobis(2,4-dimethylvaleronitrile) (V-65) were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) was purchased from Peptide Institute, Inc. (Osaka, Japan). Poly(vinyl alcohol) (PVA, degree of polymerization 500) was purchased from Katayama Chemical Industries Co., Ltd. (Osaka, Japan). Styrene, DVB and CH$_2$Cl$_2$ were purified by distillation prior to use. Other reagents and solvents were used without further purification.

**Preparation of bisphenol A di(4-vinyl benzoate) (BPA-4VB)**

The template molecule, BPA-4VB, was prepared as previously reported. Briefly, 4-VBA (2.0 g, 15.6 mmol) was dissolved in dry CH$_2$Cl$_2$ (120 mL), and 4-DMAP (1.97 g, 15.6 mmol), EDC (3.06 g, 15.6 mmol) and 2,2'-azobis(2,4-dimethylvaleronitrile) (V-65) were dissolved in 1 mL of CH$_2$Cl$_2$ wgs added to the solution; this mixture was stirred for 24 h at room temperature. The reaction mixture was washed with 10% citric acid aqueous solution and brine; then the organic phase was dried with magnesium sulfate and dried in vacuo. The product was identified by $^1$H-NMR (JNM-LA300, JEOL Ltd., Japan).

Yield: 71%, $^1$H-NMR (300 MHz, CDCl$_3$): δ = 1.72 (s, CH$_3$, 6H), 5.45 (d, H$_2$O=H$_2$O=C=CH$_2$, 2H), 5.94 (d, H$_2$O=H$_2$O=C=CH$_2$, 2H), 6.80 (q, H$_2$O=H$_2$O=C=CH$_2$, 2H), 7.14 (d, benzene, 4H), 7.29 (t, benzene, 2H), 7.54 (d, benzene, 4H), 8.16 (d, benzene, 2H)

**Droplet formation by the Y-junction microfluidic device with various flow rates of the aqueous phases**

Droplets were prepared by a Y-junction microfluidic device obtained from TOSOH Corp.; it had a flow channel with a width of 630 μm and a depth of 330 μm (Fig. 1). To examine the relationship between the sizes of droplets formed and the ratios of flow rates of aqueous and organic phases, we flowed 5 wt% PVA aqueous solutions at various flow rates from 300 to 2200 μL min$^{-1}$ controlled by a syringe pump (KDS 210, KD Scientific Inc., USA) with a 60-mL plastic syringe (Becton, Dickinson and Co., NJ). The organic phase containing styrene (300 μmol), DVB (900 μmol), and V-65 (24 μmol) dissolved in 1.2 mL of CH$_2$Cl$_2$ was flowed at a fixed flow rate of 10 μL min$^{-1}$ by a syringe pump (Bee Syringe Pump, Bioanalytical Systems, Inc., USA) with a 2.5-mL gas-tight glass syringe (Ito Corp., Japan). The sizes of droplets obtained were measured by a stereoscopic microscope (SMZ1500, Nikon Corp., Japan).

**Preparation of BPA-imprinted polymer particles (BPA-MIP)**

For the preparation of BPA-MIP, the aqueous phase and the organic phase were flowed into the Y-junction microfluidic device with syringe pumps at flow rates of 10 and 2200 μL min$^{-1}$, respectively. The obtained oil in water droplets were collected in a Schlenk flask and polymerized for 24 h at 60°C under nitrogen atmosphere. After the polymerization, the polymer particles were washed with MeOH and acetone. The shape of the particles was observed by a scanning electron microscope (VE-9800, Keyence Corp., Japan).

In order to remove the BPA moiety, we stirred the polymer for 24 h in 5 M NaOH aqueous solution containing 50% (v/v) MeOH at room temperature, followed by washing with water, 1 M HCl aqueous solution and water, successively; the material was then dried in vacuo. The removal rate of the BPA moiety was determined by the BPA in the washing solution with a Gilson HPLC system consisting of a 231XL auto-sampler (sample size, 5 μL), two 305 pumps (eluuent, MeOH/water = 50:50 (v/v); flow rate, 1.5 mL min$^{-1}$) and a 117 UV/Vis detector (detection, 254 nm). The removal rate was calculated about to be 82%.

**Adsorption experiments of BPA**

BPA-MIP (3 mg) was incubated with BPA in CH$_2$Cl$_2$/CH$_3$CN (1:1 (v/v), 1 mL) at 25°C for 24 h. Concentrations of the BPA solution were varied from 0 to 2 mM. After the incubation, the suspensions were filtered to remove BPA-MIP, and the filtrates were evaporated. The residues were redissolved in MeOH (1 mL), and the concentrations of BPA in the filtrates were determined by the Gilson HPLC system.
Evaluation as a solid-phase extraction medium

A BPA-MIP column was prepared as follows. BPA-MIP particles (412.5 mg) were stirred in MeOH, then poured into a Pasteur pipette (i.d., 5 mm) plugged by glass wool. Prior to use, the BPA-MIP column was washed with 5 mL of MeOH and 5 mL of CH₂Cl₂. BPA in CH₂Cl₂ solution (1 mL) containing 1 μmol BPA was loaded into the column. Then 1 mL of CH₂Cl₂ was poured successively; in total, 7 fractions were collected. The solvent was changed to MeOH and 1 mL aliquots of MeOH were poured; in total, 5 mL of MeOH was flowed to collect 5 fractions. These fractions were evaporated and redissolved in MeOH; then BPA was analyzed by the Gilson HPLC system.

As a reference, a BPA elution profile was examined by using only MeOH as an eluent. Prior to use, the BPA-MIP column was washed with 5 mL of MeOH. MeOH solution (1 mL) containing 1 μmol BPA was loaded into the column. Then 1 mL of MeOH was poured repeatedly and a total of 5 fractions were collected.

Results and Discussion

Effect of the flow rates on the size of droplets formed by using the microfluidic device

The size of droplets can be changed by controlling the flow rates of the aqueous and organic phases. Under the fixed flow rate of the organic phase at 10 μL min⁻¹, the size of the obtained droplets decreased by increasing the flow rate of the aqueous phase. At 1000 μL min⁻¹, the droplets size reached about 100 μm; beyond this rate, the size was not much changed (Fig. 2). We confirmed that any sizes of the droplets from ca. 250 to 90 μm can be prepared by changing the flow rate of the aqueous phase in the present microfluidic device, and we decided that the aqueous flow rate of 2200 μL min⁻¹ should be adopted for the following experiments, as the droplet size could be insusceptible by unfavorable deviation of the flow rates for practical use. After the polymerization, the resultant polymer particles were observed by a scanning electron microscope, and the size was estimated to be about 85.9 μm (n = 50; standard deviation, 9.97 μm).

Binding activity

In order to investigate the adsorption activity of BPA-MIP particles, we drew binding isotherm of BPA toward BPA-MIP particles (Fig. 3), and the binding data were transformed into a Langmuir plot. A linear plot was observed, suggesting that fairly homogeneous binding sites could be created, where BPA-MIP particles showed a Langmuir-type binding behavior with 1 to 1 stoichiometry under the conditions employed. From the Langmuir plot, a binding constant was estimated to be 4.0 × 10³ M⁻¹. This figure was comparable to that of the previously reported BPA-imprinted bulk polymer prepared by atom transfer radical polymerization with the same template.

The binding activity values of BPA-MIP towards BPA, BPB and hexestrol were compared to evaluate the selectivity of BPA-MIP. As shown in Fig. 4, BPA was bound to BPA-MIP more strongly than other two compounds. The order of binding activity was observed parallel to the size of the molecules, confirming that BPA-MIP possesses size-selective binding activity.

For the use of MIP-BPA as a medium of solid-phase extraction,
the binding activity toward BPA should be changed by switching the eluent between polar and less-polar solvents, such as MeOH and CH₂Cl₂, respectively. After packing BPA-MIP to a column, 1 mM BPA dissolved in CH₂Cl₂ (1 mL) was loaded. First, CH₂Cl₂ was used as an eluent solvent, and seven successive 1 mL aliquots of fractions were collected. Then, the solvent was changed to MeOH and five successive 1 mL aliquots of fractions were collected (Fig. 5a). Whereas most of BPA applied was not eluted during the elution by CH₂Cl₂, BPA was easily eluted with a quantitative recovery (108%), when the solvent was changed to MeOH. In order to confirm the phenomenon, we carried out the elution with only MeOH, and seven successive 1 mL aliquots of fractions were collected. As can be seen in Fig. 5b, BPA was not retained and almost all of the BPA eluted by the third fraction with 98% recovery. These results revealed that BPA-MIP particles can be applied to solid-phase extraction.

Conclusions

BPA-imprinted polymers were prepared using the Y-junction microfluidic device, which provided μm-sized spherical particles with a relatively narrow size distribution without grinding and sieving. The change of binding activity toward BPA was confirmed by switching the polar and less-polar solvents, suggesting that the BPA-imprinted microparticles could be applied to affinity-based solid-phase extraction media. Such a simple and easy way to prepare MIP microparticles could facilitate the development of further MIP technology-based affinity media in various fields of materials chemistry, biotechnology and environmental science.

Acknowledgements

The authors thank Yokkaichi Research Laboratory of TOSOH Corp. (Japan) for kindly providing the microfluidic device.

References

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