Fabrication of pH-Responsive Molecularly Imprinted Polyethersulfone Particles for Bisphenol-A Uptake

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ABSTRACT: pH-responsive molecularly imprinted particles were successfully fabricated by pore-filling poly (acrylic acid) (PAA) gels into bisphenol-A (BPA)-imprinted polyethersulfone particles. The adsorbed BPA amount (or rate) decreased after filling the PAA gels both for the imprinted and nonimprinted particles. However, it was confirmed that changing the acidity of the solution reversibly controls the rebinding ability toward BPA and that the BPA uptake of the pore-filled particles exhibited chemical valve behavior at a pH between 3 and 6. This finding can be attributed to the swelling of the PAA gels in the particles. The present methodology provides a simple way to prepare pH-responsive molecularly imprinted materials and is expandable to the imprinting of other hydrophobic molecules, such as dibenzofuran. Also, the results of this work demonstrate the potential of stimuli-responsive molecularly imprinted polymer materials as smart chemicals and as drug-delivery systems. © 2009 Wiley Periodicals, Inc. J Appl Polym Sci 113: 916–921, 2009

Key words: pH-responsive; molecularly imprinted particle; polyethersulfone; bisphenol-A adsorption

INTRODUCTION

Stimuli-responsive ("intelligent") materials (SRMs) exhibit abrupt property changes in response to small changes in external stimuli such as temperature, pH, ionic and/or solvent composition of the media, concentration of specific chemical species, electric field, and photo-irradiation.[1] Because of their intriguing properties, interest in the potential applications of SRMs in drug delivery,[2–4] biotechnology,[5–11] separation sciences,[12–16] and chemosensing[17–19] is growing rapidly. Recently, Gong et al.[1,36–39] reported a photoresponsive molecularly imprinted polymer (MIP) for the photoregulated uptake and release of caffeine, the substrate affinity of the MIP receptor sites is photoswitchable. The MIP is fabricated from an azobenzene-based functional monomer, 4-[(4-methacryloyloxy)phenylazo]. Receptor sites that are capable of recognizing specific molecular species can be conveniently imprinted into rigid polymer matrices via molecular imprinting techniques.[20,21] By incorporating SRMs, MIPs with a substrate affinity that can be switched by externally applied stimuli should be possible.

pH responsiveness is one of the most frequently adopted external stimuli for SRMs because it is convenient to apply and easy to control. Polymeric hydrogels for pH-responsive drug release have been widely studied.[3,22–25] The pH-responsive drug-delivery systems have been targeted for peroral-controlled drug delivery, taste-masking of bitter drugs, and intravascular drug release during increased blood pH in certain cardiovascular defects. Also, pH-sensitive membranes that consist of host neutral substrate and incorporated polyelectrolytes have been developed.[26–31] The host substrate, which is porous and physically and chemically stable, provides mechanical strength for the developed membranes and the polyelectrolytes (such as poly (acrylic acid) and poly (vinyl pyridine)) provide the pH sensitivity. These membranes show outstanding pH valve effect and the capability of rejecting small inorganic ions in the process of membrane separation and demonstrate a rapid and reversible response of flux to environmental pH. By combining the pH-sensitivity within MIPs, the binding and recognition property can be switched by externally applied pH stimuli.

Bisphenol A (BPA)-imprinted polyethersulfone (PES) particles and membranes were prepared in our earlier studies.[32–35] The BPA-imprinted PES was able to selectively bind and adsorb BPA. In the present study, BPA-imprinted PES particles were incorporated with cross-linked poly (acrylic acid) (PAA) gels to obtain pH-sensitive imprinted particles.

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EXPERIMENTAL SECTION

Materials and apparatus

PES (Ultrason E 6020P, CAS no. 25608-63-3) was purchased from BASF AG (Ludwigshafen, Germany) and was used to prepare the porous particles. BPA was purchased from Shanghai Chemical Reagent (Shanghai, China) and was used as the template. Dimethyl acetamide (DMAc), ethanol, acrylic acid, sodium hydroxid (NaOH), hydrochloric acid (HCl), cross-linker *N*,*N*-methylenebis (acrylamide), and initiator 2,2′-azo-bis-iso-butyronitrile were obtained from Chengdu Chemical Reagent (Chengdu, China). All the chemicals were analytical grade and used without further purification unless otherwise described. Distilled water passed through ion-exchange columns was used throughout the studies.

Preparation of BPA imprinted particles

Molecularly imprinted particles were prepared by a phase inversion technique. PES and BPA solutions were prepared with a concentration of 24 and 5 wt % in DMAc, respectively. The resultant polymer solution was vacuumized for at least 20 min. The solution was dropped into distilled water by using a 0.6-mm diameter syringe needle at room temperature to prepare particles. The particles were incubated in water for over the course of 24 h with stirring to elute the DMAc from the particles. Then, extraction of the template molecules from solidified polymers was performed by washing them with methanol, ethanol, or 1,4-butylene glycol for several days at 40°C. Using a UV–vis spectrophotometer U-200A (Shanghai Spectrum Instruments, Shanghai, China), extraction was confirmed with the disappearance of 276 nm BPA absorption in the extracted solutions. Simultaneously, PES solution with a concentration of 24 wt % was used to prepare nonimprinted PES particles in the same manner.

Preparation of pore-filled particles

To prepare pore-filled particles, nascent PES particle samples were soaked in acrylic acid aqueous solutions containing *N*,*N*-methylenebis (acrylamide) and 2,2′-azo-bis-iso-butyronitrile with a certain concentration ratios to the acrylic acid for 6–8 h. The polymerization was conducted in an oven at 65°C for 24 h to complete the reaction. After the polymerization, the particles were thoroughly extracted with boiling deionized water for 4 h to remove the unreacted monomers.

The pore-filling yield (G) was calculated as the percentage of mass increase of the substrate particle: $G\% = (m_C \times m_0)/m_0 \times 100$, where G is the mass gain, m_0 is the mass of the sample prior to the cross-linking, and m_C is the mass of the cross-linked, extracted, and then dried sample.

Scanning electron micrograph (SEM)

For the SEM observation, the particle samples were dried at room temperature. Then, the particles were quenched by liquid nitrogenous gas, cut with a single-edged razor blade, attached to the sample supports and coated with a gold layer. A scanning electron microscope (JSM-5900LV, JEOL) was used for the morphology observation of the microspheres cross-section.

Determination of ion exchange capacity (IEC)

The particles (about 0.02–0.05 g) were conditioned by repeated alternating treatments with 1*M* HCl and 1*M* NaOH, and washed in-between with water. In the final step, the particles were converted into free base form by a treatment with 1*M* NaOH followed by a thorough washing with deionized water. The particles were placed in a dilute ($\sim$ 0.05*M*) HCl solution of known volume and concentration for 12–16 h. The volume of the solution was so adjusted to contain $\sim$ 50% more HCl than the required for the theoretical ion exchange capacity calculated from the sample mass gain. The samples were removed from the solution and held above it while the particle surface was rinsed with deionized water. The samples were dried in an oven at 80°C for 48 h, weighed and marked as m_d. The residual solution was titrated with NaOH and the IEC was calculated from the following relationship:

$$\text{IEC (mequiv/g)} = \frac{V_{\text{HCl}}N_{HCl} - V_{\text{NaOH}}N_{\text{NaOH}}}{m_d} \times 1000$$

where V_{HCl} and V_{NaOH} were the volumes of HCl and NaOH solutions, respectively; and N_{HCl} and N_{NaOH} were the concentration of HCl and NaOH solutions, respectively.

MIP rebinding assay of the particles toward BPA

To study the recognition ability of the imprinted particles, we conducted binding experiments in 150 μ*M* BPA aqueous solutions with certain pH values adjusted by HCl and NaOH solutions at 20°C. To investigate the pH-responsive uptake, the particles were incubated in BPA solution as the pH was alternated between 2.5 and 7.5 for 4 h and washed in-between with water. The concentration of the BPA solution was monitored by a UV–vis spectrophotometer for 276 nm BPA absorption.

RESULTS AND DISCUSSION

The fabrication of imprinted particles

The preparing PES polymer particles were opaque in appearance and satisfactorily strong for a high-

pressure application. Figure 1 shows the SEM photographs for the cross sections of the particles. In the total cross section, many macrovoids were observed in Figure 1(a). The macrovoids distributed inside the porous PES particles. A skin layer was found, under which was a finger-like structure as shown in Figure

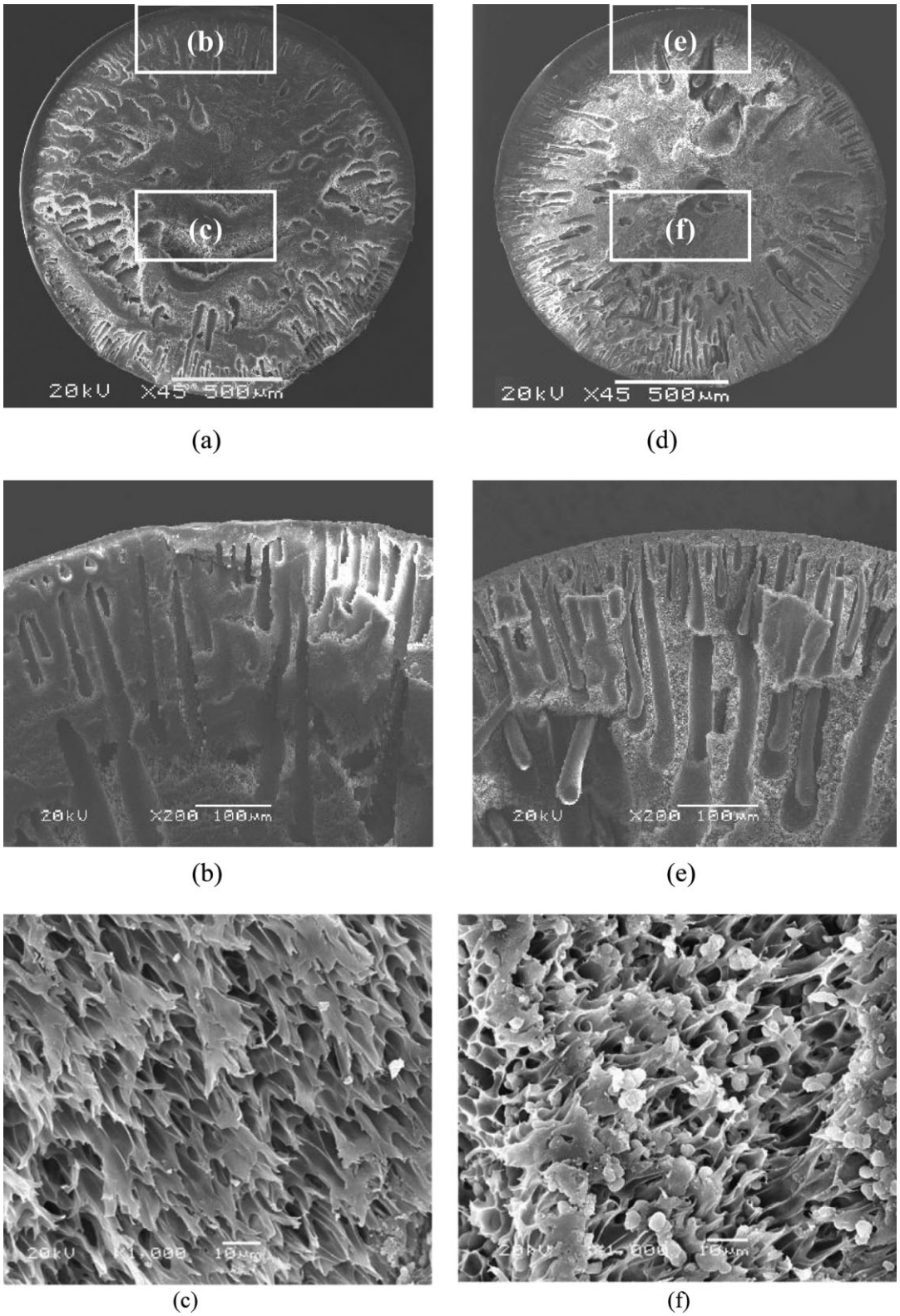


Figure 1 SEM photographs of the cross sections of the particles. (a), (b), and (c) are for the imprinted particles without PAA; (d), (e), and (f) are for the imprinted particles with pore-filling of PAA gels. Voltage: 20 kV; magnification: (a), (d) ×45; (b), (e) ×200; and (c), (f) ×1000.

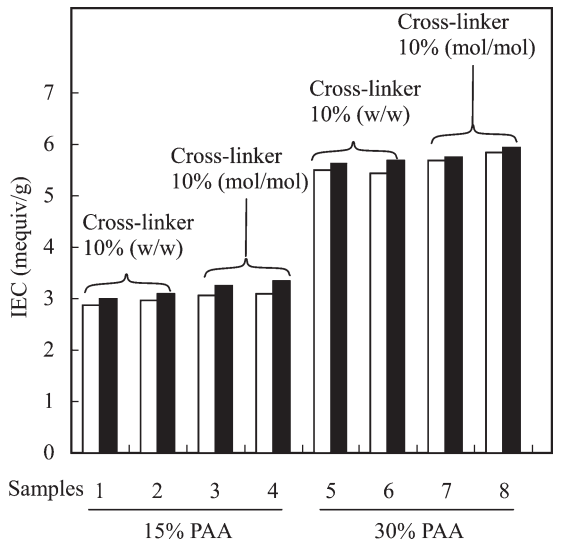


Figure 2 Titrated (open bars) and calculated (closed bars) IECs for the imprinted and nonimprinted particles prepared using different mass of cross-linker and different acrylic acid concentrations (indicating different mass gain). Samples 1, 3, 5, and 7 are for the nonimprinted particles; and samples 2, 4, 6, and 8 are for the imprinted particles.

1(b), which was magnified 200×. In this case, many small pores were observed inside the particles as shown in Figure 1(c), which was magnified 1000×. The SEM morphology suggested that the PES transformation from polymer solution to the solid state occurred quickly in poor solvent and followed by instantaneous de-mixing of PES precipitation.[32–35] Because of the poor solubility of BPA in water, the BPA remained in the solid PES microspheres when the exchange between DMAc and water proceeded. Thus, after the extraction of BPA from the solid particles, imprinting sites of the template were formed.

The pH-responsive MIP particles were prepared by pore-filling cross-linked PAA gels into the BPA-imprinted PES particles. The cross sections of the pore-filled particle are also shown in Figure 1. Comparing Figure 1(d and a; e and b; f and c), it is clearly observed that the structure of the particle has been substantially altered by the incorporation of PAA. The void volume of the modified particle is occupied, at least in part, by the pore-filling polymer. This indicates that as PAA gel is incorporated in the particles, the pore size decreases. As more PAA is incorporated, the void volume is further reduced and the pores become smaller. From the images of c and f, it suggests a reasonably uniform incorporation throughout the whole particle.

At the concentration of cross-linker ranging from 3 to 13 mol %, the mass gain increased almost linearly with the acrylic acid concentration. When the acrylic acid concentration was 30 wt %, the mass gain was ~75%. No significant difference was found for the mass of the cross-linker ranging from 3 to 13 mol % when the acrylic acid concentration was the same. However, in the absence of the cross-linker, almost no PAA gel was incorporated in the particles.

From the particle mass gain, the charge property of the particles in terms of particle IEC could be calculated. Figure 2 shows the IECs of the pore-filled particles, including the imprinted and nonimprinted particles. Figure 2 illustrates that the negative charge groups can be readily incorporated inside the neutral substrate particles (as high as 6 mequiv/g at mass gain 75% when the acrylic acid concentration was 30 wt %). The ion exchange capacities are larger than that of the reported commercial ion exchange membranes (1–2 mequiv/g) because of the larger porosity. (They can be as high as 85%, which can be calculated from the density of the polymer and the sample weight change before and after drying.) From the data, we could conclude that the amount of cross-linker (10% w/w and 10% mol/mol) used had little effect on the IEC. Furthermore, there is no significant difference for the imprinted and the nonimprinted particles due to the similar porosity.

All the titrated IECs are slightly smaller than the calculated IECs. Once the polyelectrolyte chains are dissociated and then charged, it is difficult to introduce more same charges close to one another along the chains because of the electrostatic repulsion. This suggests that, in this study when the particles were equilibrated in the NaOH solution, the ionized carboxyl groups diminished the tendency of their neighbors to ionize. Thus, the degree of dissociation was less than 100%, resulting in the IEC difference between the titrated and the calculated.

pH-responsive uptake of BPA

The effect of pH changing from 1.5 to 8 on BPA uptake to the pore-filled imprinted particles is shown in Figure 3. From the figure, the BPA uptake of the pore-filled particles exhibits chemical valve behavior

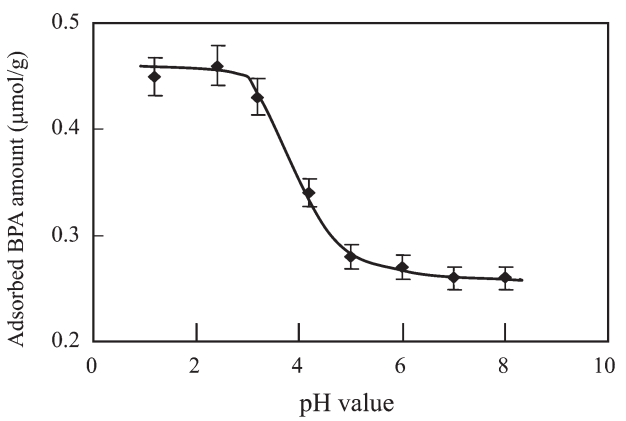
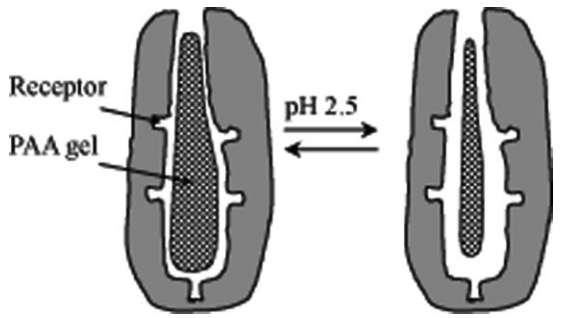


Figure 3 Adsorbed BPA amount in 4 h as a function of pH value.



Scheme 1 Switching of the substrate affinity of MIP receptors resulting from the swelling of PAA gel.

at pH between 3 and 6, and hardly changes at pH values lower than 2.5 or higher than 6.5. The adsorbed BPA amount in 4 h decreased from ~ 0.47 μmol/g at pH 2 to 0.25 μmol/g at pH 7. The chain configuration of weak polyacid is a function of pK_a of the polymer. The pK_a of PAA in solution is about 4.3–4.9, depending on the measurement method, which is consistent with that from Figure 3. Thus, in the experiments at pH lower than 3, there were at least 90% (with respect to pK_a around 4.6 from Fig. 3) of all the carboxyl groups in their unionized state. PAA gel segments coiled down resulting in pore opening. At pH values greater than 6, about 90% carboxyl groups dissociated and extended resulting in pore closing. A further decrease or increase in pH after the pH reached to 2.5 or 6.5, respectively, would not change the PAA gel configuration significantly.

For the nascent particle, the adsorbed BPA amount in the first 4 h was approximately 3 μmol/g and did not change with the variation of pH value ranging from 1.5 to 9.0. When the pH value was larger than 10, the adsorbed BPA amounts both for the pore-filled and nascent particles were sharply decreased because the pK_a value of BPA ranged from 9.59 to 11.30. Deprotonation from the hydroxyl group in bisphenol-A is negligible in the measurement conditions. Thus, we have verified that the introduction of PAA gels into the particles is essential for the creation of pH responsiveness, as shown in Scheme 1.

Furthermore, the adsorbed BPA amount to the nascent particles was larger than that to the PAA filled particles owing to the fact that even when the polymer chains are in their compact form (at low pH), the gel still swells in the substrate particle due to the volume exclusion effect.

The particle pH reversibility was evaluated by the BPA uptake at pH 2.5 and 7.5 in solutions. The adsorbed BPA rate is presented in Figure 4. Each experimental run involved 10 min of equilibration in the buffer solution followed by 4 h of adsorption, and then the adsorbed rate was calculated as μmol/g per hour. Because it took ~ 40 h to reach the equilibrium adsorption amount from the isotherm adsorption curve, the BPA uptake rate was calculated from the adsorbed amount divided by 4 and was regarded as the average rate within 4 h. From Figure 4, the BPA uptake is reversible as the buffer was alternated for the gel-filled particles including the imprinted and nonimprinted [as shown in Fig. 4(a)]. However, for the nascent particles, the adsorbed BPA rate gradually decreased even though the buffer was alternated [as shown in Fig. 4(b)]. The BPA adsorbed rate was lower for the gel filled particles than that for the nascent particles because the adsorbed amount for the pore-filled particles was smaller as mentioned previously.

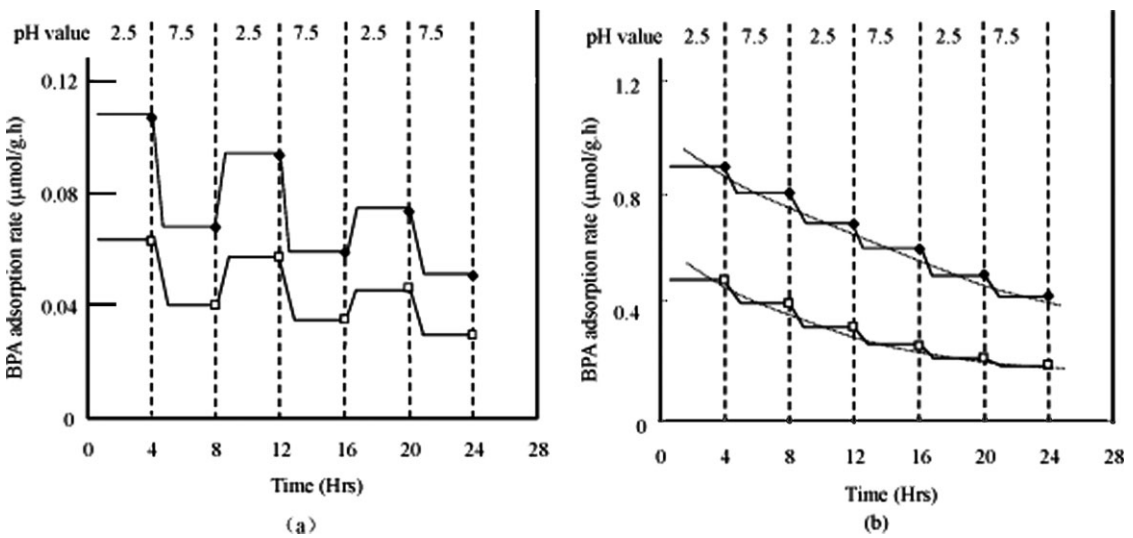


Figure 4 The BPA adsorbed rate as the solution was exchanged between pH 2.5 and 7.5 with 10-min equilibration wash followed by 4-h adsorption. (◆) For the imprinted particles; (□) for the non-imprinted particles. (a) For the gel filled particles; (b) for the nascent particles.

The adsorbed BPA rate gradually decreased with time even at the same pH value for all the particles. This result is easy to understand from the adsorption kinetics. The adsorbed rate will become 0 when the adsorption reaches equilibrium. The recognition coefficient, which can be defined as the ratio of the BPA binding amount to the imprinted particles to the amount to the nonimprinted particles, was used to evaluate the recognition ability. And the recognition coefficients were ~ 1.8 both for the pore-filled and the nascent particles.

The ability of the pore-filled particles to bind BPA was studied with a Scatchard analysis, and batch-type rebinding assays were conducted. From the plots of $[B]_{bound}/C$ versus $[B]_{bound}$, the K_D, an average equilibrium dissociation constant can be calculated from the negative slope and the B_{max}, the number of the binding sites can be obtained from the intercept. In the resultant plots, two straight-line regions were obtained for the imprinted particles and only one straight line was obtained for the nonimprinted particles. It is reasonable to assume that two different sites (specific and nonspecific binding sites) for the BPA molecules existed in the BPA imprinted particles.

CONCLUSIONS

In conclusion, the work presented herein has demonstrated for the first time a novel strategy to create pH-responsive molecularly imprinted particles from two steps: first, BPA-imprinted particles were prepared by use of a phase separation technique and then the particles were pore-filled with PAA gels. The adsorbed BPA amount (or rate) decreased after filling the PAA gels. However, it was confirmed that changing acidity of the solution reversibly controls the rebinding ability toward BPA. We expect that the present system is expandable to the imprinting of other hydrophobic molecules, such as dibenzofuran. And we believe that the present methodology is a promising way to develop environment friendly separation materials, human-body-friendly drug delivery systems, etc. in the near future.

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