

# Separation of Hydroxybenzoic Acid Isomers Using the Molecular Imprinting Technique

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**ABSTRACT:** Molecular imprinting polymers (MIPs) for salicylic acid (SA), 3-hydroxybenzoic acid (3HBA), and 4-hydroxybenzoic acid (4HBA) were synthesized using styrene and 4-vinylpyridine (4-VPy) as functional monomer and divinylbenzene (DVB) as crosslinker. The adsorption characteristics of hydroxybenzoic acid (HBA) isomers on each MIP were investigated. The materials used for the polymerization of each 3HBA and 4HBA-MIPs were adsorbed relatively well. This verifies that the MIPs that can adsorb template selectively were synthesized. However, SA-MIP had no molecular imprinting effect. SA has intra-

molecular hydrogen bond and it is difficult to adsorb on recognition site of SA-MIP, because its structure differs from that of recognition site of SA-MIP. It indicates that SA-MIP had no recognition effect. 1,2,3,4-Tetrahydro-1-naphthol (THN)-MIP was synthesized, which is similar to SA with intramolecular hydrogen bond. SA was separated selectively using THN-MIP. © 2007 Wiley Periodicals, Inc. *J Appl Polym Sci* 105: 2824–2829, 2007

**Key words:** molecular imprinting polymer; hydroxybenzoic acid isomers; separation

## INTRODUCTION

The molecular imprinting technique has focused on one of the potential methods to create molecular recognition sites having high selectivity to the target molecule into polymeric materials, which can be employed as stationary phases in chromatography, sensors, membranes, etc.<sup>1–7</sup> The molecular imprinting polymer (MIP) is synthesized generally by the copolymerization of functional vinyl monomers with a divinyl monomer as a crosslinker in the presence of template.

By using the covalent bonding procedure proposed by Wulff,<sup>8</sup> a template–monomer complex is formed by a covalent bond and polymerized. Thus, selective breakage of the covalent bond leads to a polymer network capable of recognizing the imprinted template.

The second technique named noncovalent bonding is proposed by Mosbach,<sup>9</sup> and it is very easy to carry out. In this method, a highly crosslinked polymer interacts with the template molecule simply through electrostatic interaction, hydrogen bonding, or similar noncovalent bonds. After the retained template is washed out, the polymer shows a formation of com-

plementary sites with significant molecular recognition properties.

In general, most of the research on MIP have used very high portion of crosslinking monomer to maintain the three-dimensional shapes for the template.<sup>10–14</sup> As a consequence of the use of high concentration of crosslinking monomer, an extremely rigid polymer were obtained. The rigidity makes MIP very difficult to remove the template. Thus, it is used as a fine powder to extract template effectively. The number of molecular recognition sites becomes much smaller compared to their expected sites from the amount of the template used, because some binding sites in the powder are buried in the three-dimensional network structure of polymers.<sup>15</sup> Practically, only the binding sites on the powder surface are used for the rebinding of guests due to the difficulty of the diffusion into the rigid polymer. Some MIPs can be soluble or swollen in organic solvents depending on the content of crosslinking agents. If a MIP is soluble or highly swollen in the process of template extraction, the MIP may lose binding selectivity to the template because the imprinted three-dimensional spaces can be destroyed by the softness, while all the templates buried inside the powder can be removed. When an appropriate amount of crosslinker is used, the soft MIP can be swollen reversibly in an organic solvent through the extraction process maintaining the original imprinting size and selectivity to template. The MIP removed the template

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**TABLE I**  
The Components of the Reaction Mixture for the Preparation of the Imprinted Polymers

MIPs	Styrene	4-Vinylpyridine	Template (SA, 3HBA, 4HBA)	Divinylbenzene	AIBN (wt %)
Template-MIP	30	1	1	0.5	0.2
				0.8	
				1.0	
				1.5	
Control polymer	30	1	–	0.5	0.2
				0.8	
				1.0	
				1.5	

through the reversible swelling may have high uptake to a guest molecule.

This paper appears as a sequence of the studies previously performed on toluic acid isomers by our research group<sup>16</sup> and now extended to MIPs for HBA isomers using small amount of crosslinker.

## EXPERIMENTAL

### Materials

Salicylic acid (SA), 3-hydroxybenzoic acid (3HBA), 4-hydroxybenzoic acid (4HBA), 4-vinylpyridine (4-VPy), styrene, 1,2,3,4-tetrahydro-1-naphthol (THN), 1-naphthol (NA), 1-naphthoic acid (NAA), divinylbenzene (DVB), NaOH, and toluene were purchased from Aldrich Chemical Company (Milwaukee, WI, USA). Azobis(isobutyronitrile) (AIBN) and ethanol were obtained from Junsei Chemical (Japan). Styrene, 4-vinylpyridine, and divinylbenzene were distilled under vacuum to remove the inhibitors before polymerization. All solutions were prepared in nano-pure water.

### Preparation of molecular imprinting polymers

Each isomer of hydroxybenzoic acid (SA, 3HBA, and 4HBA), THN, NA, and NAA was mixed with 4-vinyl pyridine to form a 1:1 complex, and the complex was mixed with styrene, DVB as a crosslinker, and initiator AIBN. The mixing composition is shown in Table I and Table II. The resulting solution was copolymerized at 65°C for 48 h under N<sub>2</sub> atmosphere. The control polymers were prepared without template only for comparing the substrate uptake to corresponding MIPs.

### Removal and uptake of templates

The MIPs without crosslinker were ground to pass through 65 mesh sieve. The fine powder was obtained in nano-pure water. The removal of the templates was conducted in ethanol with Soxhlet. Then the MIPs were cleaned with 0.1M NaOH and water alternately until the template was not detected by UV spectrophotometry.

The MIPs with crosslinker were swollen in toluene at first, and then ethanol, a good solvent for HBA isomers, was added with stirring to remove the template effectively. After its removal, the MIPs were ground to pass through 65 mesh in water and dried.

### Adsorption evaluation

The uptake was measured by using a spectrophotometer after adding, respectively, 20 mg (SA-MIP), 30 mg (3HBA-MIP), and 10 mg (4HBA-MIP) was added to 10 mL SA, 10 mL 3HBA, and 20 mL 4HBA aqueous solution containing  $5.9 \times 10^{-4}$ ,  $1.4 \times 10^{-4}$ , and  $9.0 \times 10^{-4}$  mol/L of the guest molecules.

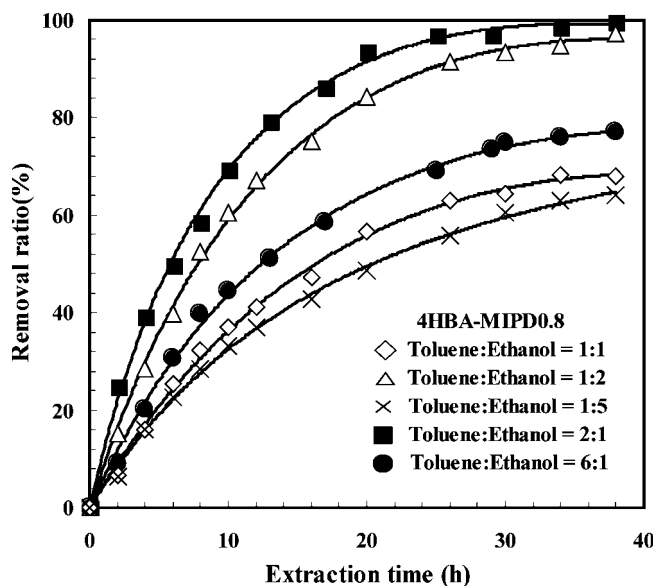
The amount of HBA bound to the imprint polymer uptake ( $\mu\text{mol/L}$ ) was calculated by the following Eq. (1):

$$\text{Uptake } (\mu\text{mol/L}) = (C_0 - C_t)V/W \quad (1)$$

where  $C_0$  and  $C_t$  were the SA, 3HBA, and 4HBA concentration (mol/L), measured at initial and post interval time (hour) for equilibrium. Symbols  $V$  and  $W$  denote the volume of the SA, 3HBA, and 4HBA solution, and the weight of dry polymer used for the adsorption experiment, respectively.

**TABLE II**  
The Components of the Reaction Mixture for the Preparation of NA-MIP, NAA-MIP, and THN-MIP

MIPs	Styrene	4-Vinylpyridine	Template	Divinylbenzene	AIBN (wt %)
NA-MIP	30	1	1	0.8	0.2
NAA-MIP	30	1	1	0.8	0.2
THN-MIP	30	1	1	0.8	0.2

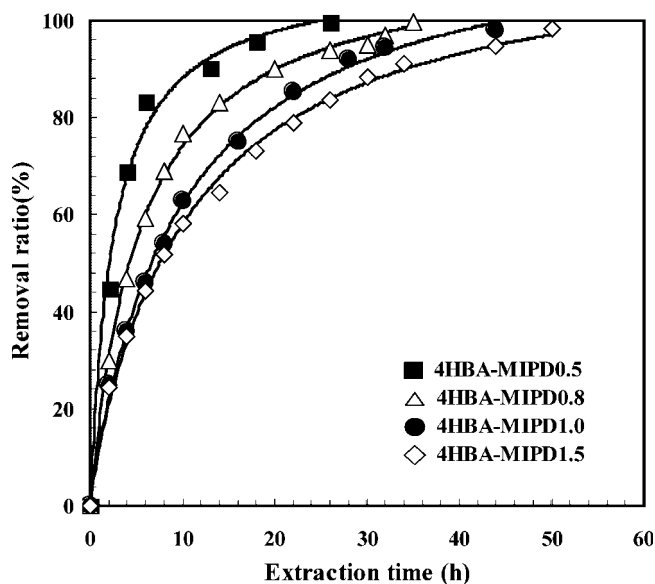


**Figure 1** Removal ratio of 4-hydroxybenzoic acid template depending on various mixing ratio of toluene/ethanol (v/v).

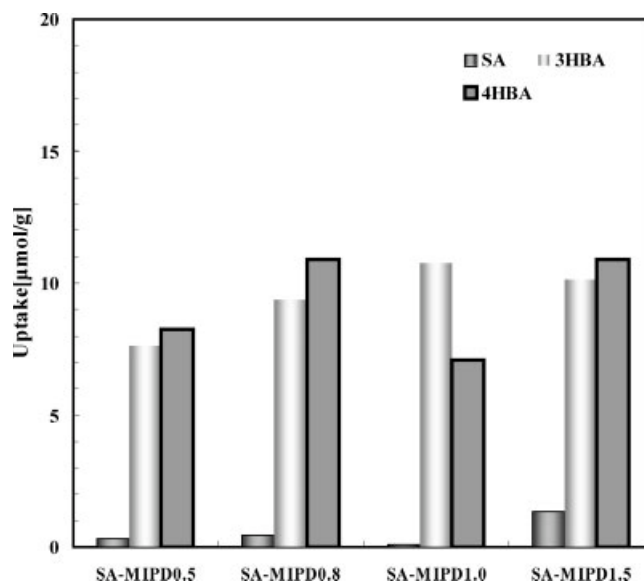
Selective factor ( $\alpha$ ) of the imprinted polymer was the relative value of SA, 3HBA, and 4HBA adsorbed to the imprinted polymer compared with that of SA, 3HBA, and 4HBA template, and the selective factor was calculated by the following formula (2):

$$\alpha = \text{Uptake} / \text{Uptake}_{\text{SA, 3HBA, and 4HBA}} \quad (2)$$

where Uptake ( $\mu\text{mol/g}$  polymer) was binding amounts of SA, 3HBA, and 4HBA and  $\text{Uptake}_{\text{(SA, 3HBA, and 4HBA)}}$  for SA, 3HBA, and 4HBA.



**Figure 2** Removal curve of 4-hydroxybenzoic acid template depending on the amount of DVB in a mixed solvent of toluene/ethanol = 2 : 1 (v/v).

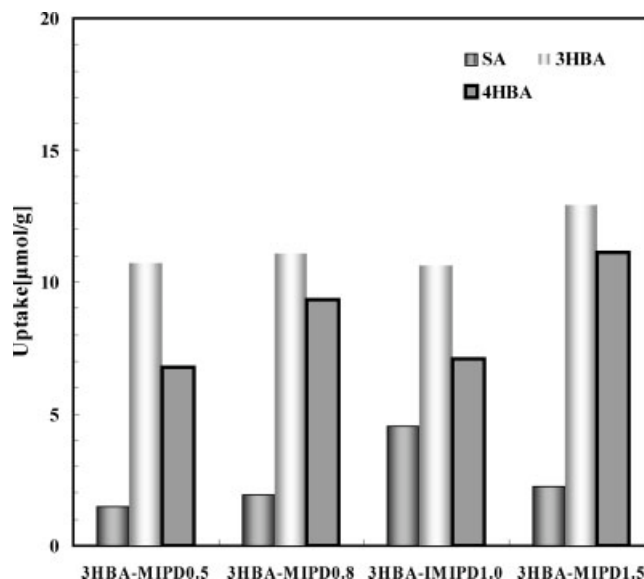


**Figure 3** Uptakes in term of crosslinker content for SA-MIP.

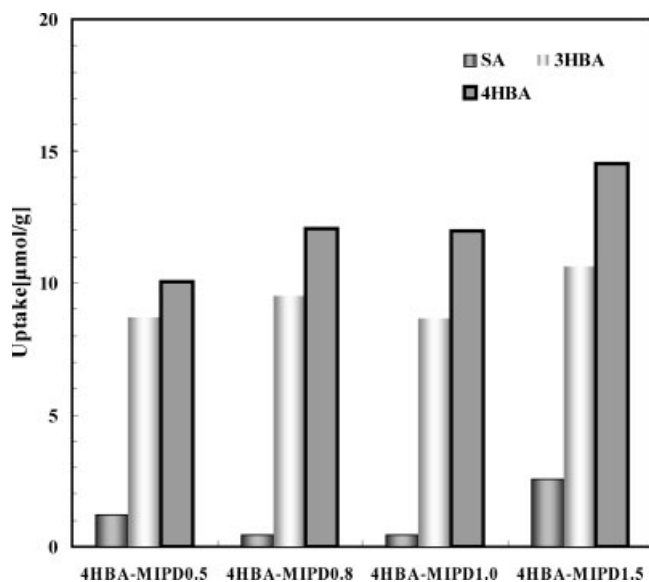
## RESULTS AND DISCUSSION

### Removal of the template

The swelling process was performed to remove the template of MIPs containing the crosslinker (DVB). The template was removed by using a mixture of toluene/ethanol ( $x:y$ ), which we found as an adequate solvent for template removal. Figure 1 shows the percentage of removal ratio. The highest value was found when the mixture with toluene/ethanol = 2:1 was used among the mixtures with the various ratios of toluene and ethanol.



**Figure 4** Uptakes in term of crosslinker content for 3HBA-MIP.



**Figure 5** Uptakes in term of crosslinker content for 4HBA-MIP.

In Figure 2, various MIPs were synthesized for each template by varying the quantity of the crosslinker (DVB) into 0.5, 0.8, 1.0, and 1.5 mol. The variation, with time, of the amount of the template removed using the mixed solvent with toluene/ethanol = 2:1 is given in Figure 1. The removal of template got easier as the amount of crosslinker became smaller, but the removal rate of the template reached the equilibrium after 50 h.

#### Effect of molecular imprinting polymers

Figures 3–5 shows the adsorption quantities of HBA-MIPs synthesized with different amounts of the crosslinker. All the 3HBA-MIP with the different mole ratio of the crosslinker showed selective separation. The adsorbed quantity increased a little as the mole ratio of the crosslinker got larger, but the adsorbed amount will decrease because the removal of template becomes harder due to little occurrence of the swelling if the quantity of the crosslinker increases above certain quantity. The quantity of the 4HBA adsorbed to 3HBA-MIP was always larger

than that of SA. The molar volume of SA is greater than the recognition site as a result of intermolecular hydrogen bond in the molecules, in which —OH of ortho site combines with —COOH.

The 4HBA has smaller steric hindrance since it has linear structure. The adsorbed quantity of 4HBA will thus be larger. Figure 5 shows the adsorbed quantity of 4HBA-MIPs synthesized with different mole ratio of the crosslinker. The 4HBA-MIP also gives good selective adsorption irrespective of the amount of the crosslinker in the mole ratio range of the used crosslinker. 3HBA-MIPs and 4HBA-MIPs with the 1.5 mol mole ratio of the crosslinker have the largest adsorption quantity. In the range of the used mole ratio of the crosslinker, the template seems to be easily removed not only from the surface but also from the interior through the swelling process.

Table III shows the amount of adsorbed SA, 3HBA, and 4HBA for the each MIPs synthesized with the 0.8 mol mole ratio of the crosslinker and control polymer. Each MIPs adsorbed selectively the template that was used to synthesize each MIPs when it is compared with the control polymer. However, SA-MIP dose not adsorb SA. SA-MIP rather adsorbed a large amount of 3HBA and 4HBA. This shows that SA cannot be separated by using SA-MIP. This is because SA forms an intramolecular hydrogen bond within itself.

#### Separation of salicylic acid

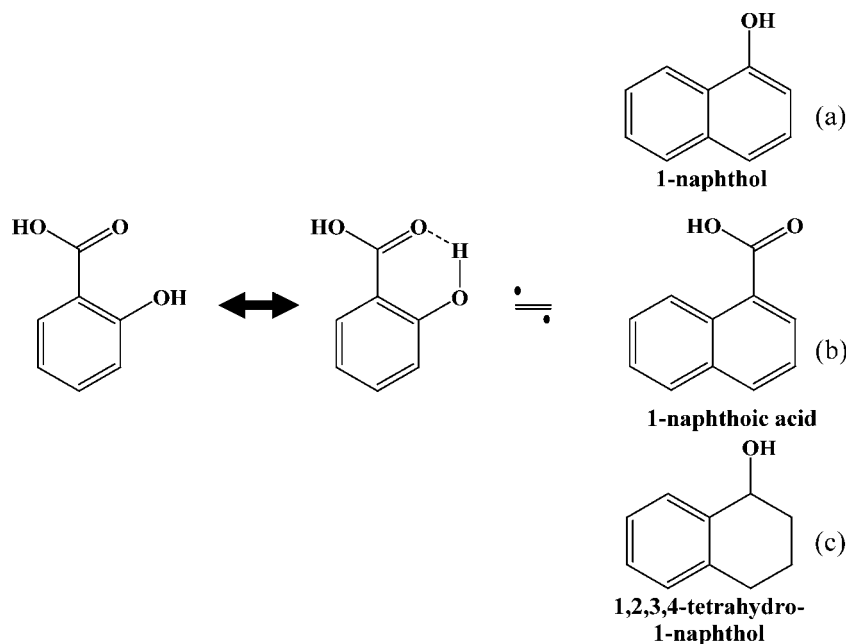
SA-MIP had no recognition ability even if the quantity of the functional monomer was increased to separate selectively SA. Therefore, MIPs was synthesized by using THN, NA, and NAA with the structure similar to the SA having intramolecular hydrogen bonding (Fig. 6).

Figure 7 shows the uptake for HBA isomers to THN-MIP, NA-MIP, and NAA-MIP. These three MIPs give much higher uptake than SA-MIP for SA. However, NA-MIP and NAA-MIP adsorbed more other isomers than SA, showing no selective separation effect.

Figure 8 shows the uptake of HBA isomers for SA-MIP and THN-MIP. THN-MIP adsorbed more SA than SA-MIP, and separated selectively other iso-

**TABLE III**  
Uptake and Selectivity of Various MIPs with the Corresponding Control Polymer

MIPs	Guest template	Uptake (μmol/g)			Uptake ratio to control			Selectivity		
		SA	3HBA	4HBA	SA	3HBA	4HBA	SA	3HBA	4HBA
Template-MIP0.8	SA	0.45 ± 1.4	9.4 ± 1.1	10.9 ± 0.78	0.28	5.22	3.03	1	0.56	0.62
	3HBA	1.96 ± 1.17	16.91 ± 1.91	10.1 ± 1.89	1.23	9.39	2.81	4.36	1	0.57
	4HBA	0.45 ± 0.86	9.5 ± 1.07	17.6 ± 1.57	0.28	5.28	4.89	1	0.56	1
	CPD0.8	1.6 ± 0.38	1.8 ± 0.31	3.6 ± 0.44	1	1	1	–	–	–



**Figure 6** Schematic diagrams for the intramolecular hydrogen bonding in SA. (a), (b), and (c) are materials having similar structure.

mers. This result shows that using THN-MIP is the most effective way to separate SA selectively.

### CONCLUSIONS

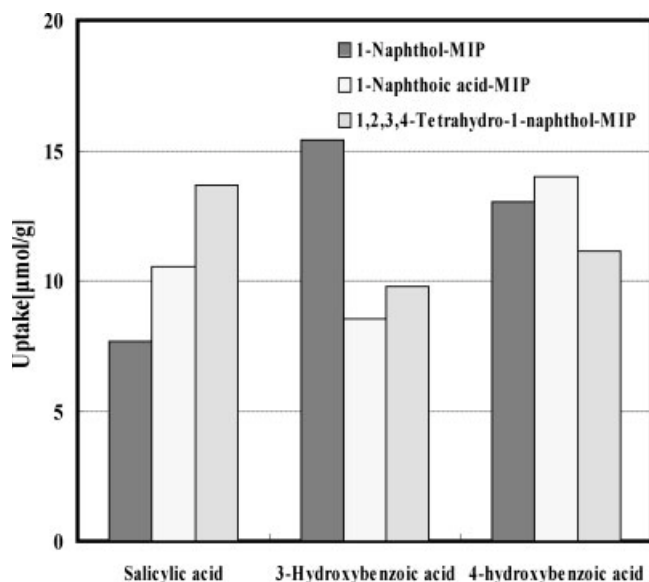
The adsorption properties of each template were investigated for MIPs synthesized by using SA, 3HBA, and 4HBA as template.

The swelling process was performed to remove the template of MIPs. The removal rate of template was highest when the mixture with toluene/ethanol

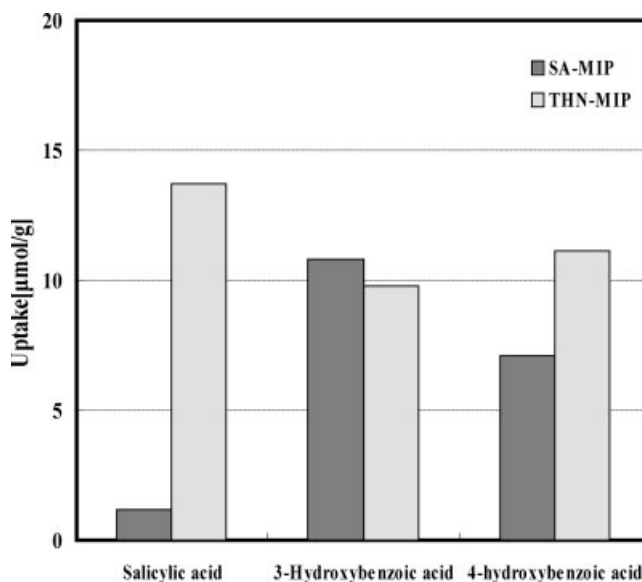
= 2:1 was used among the mixtures with the various ratios of toluene and ethanol.

As compared with control polymer that did not imprint template, the adsorption selectivity of SA-MIP, 3HBA-MIP, and 4HBA-MIP was high, but SA-MIP had no selectivity.

The data discussed in this article demonstrate that 3HBA and 4HBA-MIPs with different mole ratio of the crosslinker (0.5, 0.8, 1.0, and 1.5) exhibit selective separation. SA-MIP had no recognition ability even if the quantity of the functional monomer was



**Figure 7** Uptakes of NA-MIP, NAA-MIP, and THN-MIP.



**Figure 8** Uptakes of SA-MIP and THN-MIP.

increased to separate selectively SA. Therefore, MIPs were synthesized by using THN, NA, and NAA with the structure similar to the SA having hydrogen bonding within the molecule. Thus, only THN-MIP has selective recognition ability for SA.

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