Molecular Imprinting Polymers for the Separation of Toluic Acid Isomers

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ABSTRACT: Molecular imprinting polymers (MIPs) were prepared with styrene, 4-vinyl pyridine, and divinylbenzene for the separation of toluic acid isomers. The uptake and selectivity were investigated, with respect to how they were governed by the swelling degree of a soft MIP that contained a small amount of crosslinker. The optimum swelling range led to high uptake and the easy removal of the template without sacrificing the selectivity, which was controlled by the shape and size of the imprinting cavity under the same functional monomer, to the guest molecule. The original imprinting cavities were reversibly maintained within the range of 200% swelling and shrinking of MIPs throughout the template extraction. © 2005 Wiley Periodicals, Inc. J Appl Polym Sci 96: 650–654, 2005

Key words: molecular imprinting; molecular recognition; separation technique

INTRODUCTION

The molecular imprinting technique is a potential method for creating molecular recognition sites with high selectivity to the target molecule in polymeric materials, which can be employed as stationary phases in chromatography, sensors, membranes, and so forth.[1–8] A molecular imprinting polymer (MIP) is generally synthesized through the copolymerization of functional vinyl monomers with a divinyl monomer as the crosslinker in the presence of a template. After the removal of the template with the appropriate solvents, the resulting polymer has a memory for selectively recognizing the guest molecule, used as the template due to the shape of the footprint-like vacant spaces. The formation of host–guest complexes is driven by intermolecular interactions involving ionic pairs, hydrogen bonding, van der Waals forces, and so forth and is also affected by the physically imprinted three-dimensional nest size and shape of the template.[1,9–15] Functional vinyl monomers control the interactions with the template, and the crosslinking monomer works as the base, maintaining three-dimensional shapes for nesting the guest in the MIPs. In general, most research on MIPs has used very large portions of the crosslinking monomer to maintain the three-dimensional shapes for the template.[16–21] They are very rigid because of the high concentration of the crosslinking monomer. The rigidity makes MIPs very difficult to remove from the template. Thus, they are used as fine powders for the effective extraction of the template. The number of molecular recognition sites becomes much smaller than the number of sites expected from the amount of the template used because some binding sites in the powder are buried in the three-dimensional network structure of the polymers.[22] Practically only the binding sites on the powder surface are used for the rebinding of guests because of the difficulty of diffusion into the rigid polymer.

Some MIPs can be soluble or swollen in organic solvents according to the concentrations of the crosslinking agents. If an MIP is soluble or highly swollen during 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, whereas all the templates buried inside the powder can be removed. When an appropriate amount of the crosslinker is used, a soft MIP can be swollen reversibly in an organic solvent through the extraction process, and the original imprinting size and selectivity to the template can be maintained. An MIP removed from the template through reversible swelling may have high uptake to a guest molecule.

In this study, two types of MIPs for toluic acid isomers were prepared with and without the crosslinker. In MIPs without the crosslinker, the template was extracted from the powder state according to a generally known method with a solvent that was a nonsolvent for the polymer but a good solvent for

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TABLE I
Composition of MIPs with and without DVB as a Crosslinker

MIP	Styrene (mol)	4-VP (mol)	DVB (mol)	Template (mol)	AIBN (wt %)
IPSV	30	1	—	1	0.1
Control 1	30	1	—	—	0.1
IPD 0.5	30	1	0.5	1	0.1
Control 2	30	1	0.5	—	0.1

the template. MIPs with a relatively small amount of the crosslinker were swollen with a mixed solvent for the removal of the template to determine the shape and size effects of imprinting sites. We report the changes in the rebinding and selectivity properties for the two types of MIPs, considering how recognition memory can be changed through the swelling in a solvent.

EXPERIMENTAL

Materials

Toluic acids (*o*-, *m*-, and *p*-isomers), 4-vinyl pyridine (4-VP), styrene, divinylbenzene (DVB), NaOH, and toluene were purchased from Aldrich (Milwaukee, WI). Azobisisobutyronitrile (AIBN) and ethanol were obtained from Junsei Chemical (Tokyo, Japan). The monomers were used after the stabilizers were removed, but toluic acids were used without further purification. All solutions were prepared in nanopure water.

Preparation of MIPs

Each isomer of toluic acids was mixed with 4-vinyl pyridine to form a 1:1 complex, and the complex was mixed with styrene, the crosslinker DVB, and the initiator AIBN. The mixing compositions are shown in Table I. The resulting solution was copolymerized at 65°C for 48 h under an N_2 atmosphere. The control polymers were prepared without the template for a comparison of the substrate uptake to that of the corresponding MIPs.

Removal and uptake of templates

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

The MIPs with the crosslinker were swollen in toluene at first, and then ethanol, a good solvent for toluic acids, was added with stirring to extract the template effectively. After its removal, the MIPs were

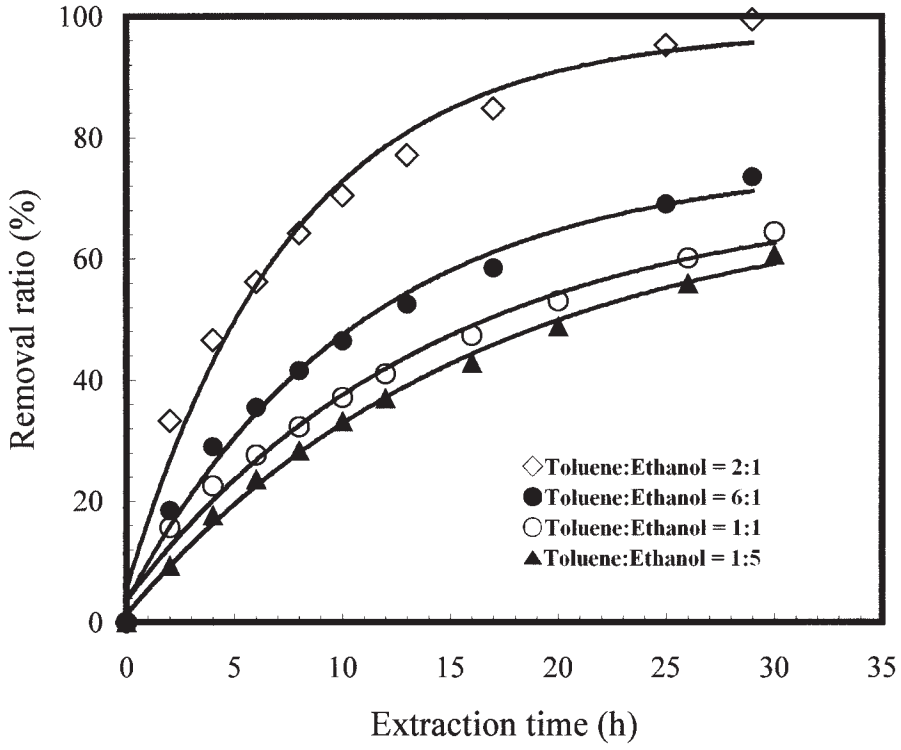

Figure 1 Template removal profiles of *o*-toluic acid imprinted polymers containing 0.5 mol of DVB (*o*-IPD 0.5) with various mixing ratios of toluene to ethanol (v/v).

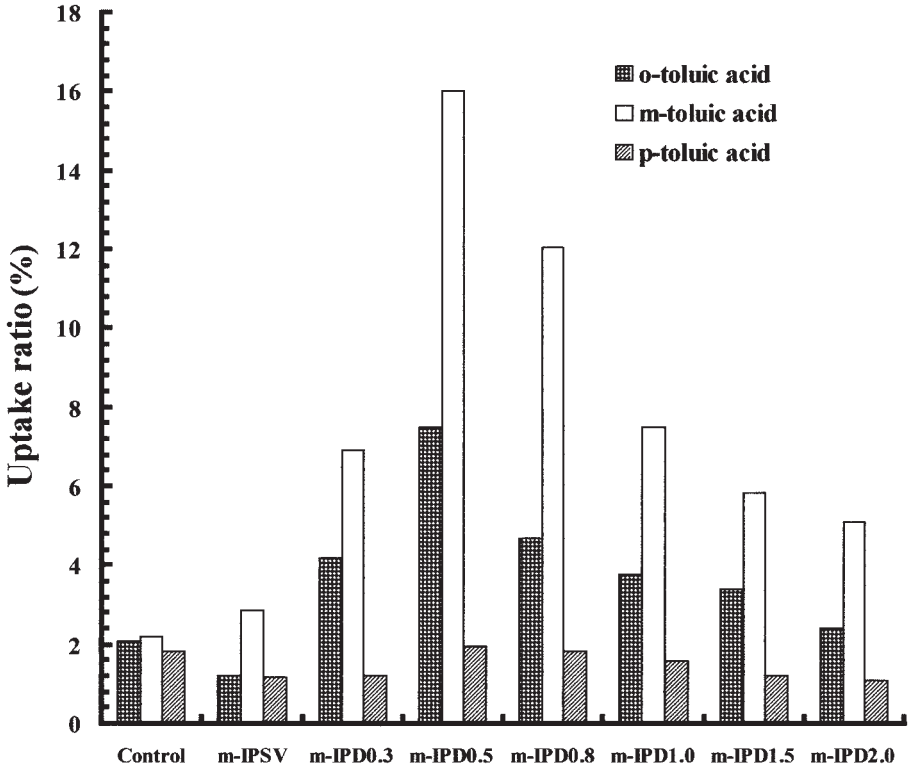

Figure 2 Comparison of the uptake ratios, with respect to the crosslinker content, for *m*-toluic acid imprinted polymers. Numeric value in each IPD represents molar content of DVB.

ground to pass through a 65-mesh (212 μm) sieve in water and dried.

The uptake was measured with a UV spectrometer after 50 mg of a dried MIP fine powder was added to a 10-mL aqueous solution containing 3.5×10^{-6} mol of the guest molecules. The MIP (50 mg) contained 1.5×10^{-6} mol of the template.

RSULTS AND DISCUSSION

Template removal from MIPs

To investigate the maintenance of the three-dimensional structure for the recognition sites in MIPs, we extracted the templates with a mixed solvent of ethanol and toluene in the swollen state of the polymer. After the polymer swelled in toluene, ethanol was added to remove the template. The removal profiles of *o*-toluic acid from the corresponding MIPs containing 0.5 mol of DVB as a crosslinker *o*-isomer imprinted polymer using 0.5 molar DVB (*o*-IPD 0.5) are shown in Figure 1 for various mixing ratios of toluene and ethanol. From the swollen MIPs, the templates could be dissolved easily in a solvent in general, in comparison with the rigid state, because of the increase in the freedom for diffusion. However, because ethanol was not a solvent for the polymer, the swollen MIPs in toluene shrank with the amount of ethanol added. The higher the ethanol content was, the more the polymer shrank. Thus, an optimum solvent mixing ratio for toluic acid extraction existed. Within our experimental range, a 2:1 ratio for toluene to ethanol was optimal. Therefore, the removal of templates was conducted in a 2:1 mixed solvent.

MIPs without the crosslinker were treated with ethanol with Soxhlet for 12 h at room temperature for one extraction. For the removal of 90% of the template, five extractions were needed.

Effect of the DVB concentration in MIPs

The amount of crosslinker can control the degree of MIP swelling. The swelling can cause the recognition sites in MIPs to expand and to reduce the selectivity because the three-dimensional imprinting structure can be distorted by the swelling and shrinking in the template extraction. The main purpose of MIP synthesis is to effectively separate a target molecule with high selectivity of the imprinted cavity. Thus, the original size of the imprinted cavity has to be maintained so that it is not distorted in the template extraction process. Most experiments have used very large amounts of the crosslinker to maintain the cavity and control the selectivity to the target.[23] However, it can be very difficult to remove the template because the high rigidity of MIPs makes it hard for a solvent molecule to penetrate the polymer and to diffuse the

TABLE II
Degree of Swelling and Extraction Time of *m*-Toluic Acid Imprinted Polymers in Terms of the Amount of Crosslinker

MIP	Degree of swelling (%) in toluene/ethanol (2:1)	Extraction time (h)[a]
m-IPD 0.3	255.8	20
m-IPD 0.5	164	30
m-IPD 1.0	108	50
m-IPD 2.0	44	70

[a] Removal time for up to 95% of the template in toluene/ethanol (2:1).

template out. In a swollen, soft MIP with a small amount of the crosslinker, it is easier to remove the template than in a rigid solid, but the selectivity is sacrificed if the cavities are distorted by the swelling. The uptakes changes are shown in Figure 2 versus the amount of DVB for *m*-toluic acid imprinted MIPs. *m*-IPD 0.5 is an *m*-toluic acid imprinted polymer containing 0.5 mol of DVB (see Table I). All the *m*-toluic acid imprinted MIPs showed the highest uptake for *m*-toluic acid of the three isomers, regardless of the crosslinker amount, and this was caused by the general character of the MIPs. As the DVB concentration increased, the uptake decreased. This was the reason that the MIPs were more rigid with higher concentrations of DVB. The rigidity made the diffusion of guest molecules into the MIPs difficult. However, the rigid MIPs might have had higher selectivity to the imprinted isomer than to the other isomers because the rigidity could maintain the original imprinted cavity. The best MIPs must have high amounts of uptake and selectivity.

The degree of swelling and the extraction time in a mixed solvent (2:1 toluene/ethanol) are shown in Table II. The degree of swelling was calculated from the change in the weight before and after dipping in a solvent to the dry weight. The extraction time was for up 95% removal of the template. For *m*-IPD 0.3, the time for template extraction was relatively short because of its high degree of swelling. The degree of swelling of *m*-IPD 2.0, with 2 mol of DVB, was drastically reduced, and removing the template took a long time. The degree of swelling could be measured to determine the expansion range of recognition cavities without the selectivity being sacrificed. With 0.5 mol of DVB, *m*-IPD 0.5 showed high uptake and selectivity, as shown in Figure 2, although the extraction process was conducted in the swollen state. The result was that the swelling of *m*-IPD 0.5 might not sacrifice the selectivity in comparison with *m*-isomer imprinted polymer using styrene and 4-vinyl pyridine without crosslinker (*m*-IPSV), which was extracted in the rigid state. The uptake of each isomer reached the highest level for *m*-IPD 0.5 and then slowly decreased with increasing DVB concentration.

Uptake and selectivity

The uptake and selectivity were investigated for MIPs with and without the crosslinker (Table III). The selectivity is defined as the ratio of the uptake of a guest molecule to the uptake of the template in the corresponding MIP. The experiments were conducted in a 10-mL aqueous solution containing 3.5×10^{-6} mol of the guest molecule because 50 mg of an MIP contained 1.5×10^{-6} mol of the template. The maximum uptake was 300×10^{-6} mol/g of MIP, which was calculated from the amount of the template added to the MIP, regardless of the physical sorption of the polymer. IPSV without the crosslinker was treated in a powder state in a nonsolvent for the polymer but a solvent for the templates. The uptake of IPSV (60 mesh) was varied from 6 to 9.3

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

		Uptake (μmol/g)			Uptake ratio to Control			Selectivity		
MIP	Template	*o*-Toluic acid	*m*-Toluic acid	*p*-Toluic acid	*o*-Toluic acid	*m*-Toluic acid	*p*-Toluic acid	S/S_0	S/S_m	S/S_p
IPSV	*o*-Toluic acid	9.34 ± 0.62	2.12 ± 0.83	2.74 ± 0.28	1.8	0.37	0.65	1	0.25	0.46
	m-Toluic acid	3.6 ± 1.02	8.4 ± 1.2	3.4 ± 0.27	0.7	1.75	0.81	0.39	1	0.57
	p-Toluic acid	5.0 ± 0.06	4.7 ± 0.28	6 ± 0.49	0.96	0.98	1.43	0.53	0.56	1
	Control 1	5.2 ± 0.06	4.7 ± 0.28	6.0 ± 0.49	1	1	1	—	—	—
IPD	*o*-Toluic acid	29.8 ± 1.1	11.8 ± 1.4	3.37 ± 0.57	4.92	1.84	0.62	1	0.25	0.19
	m-Toluic acid	22.0 ± 1.8	47.06 ± 1.8	5.72 ± 0.72	3.66	7.32	1.06	0.74	1	0.32
	p-Toluic acid	9.57 ± 1.03	11.4 ± 1.2	17.77 ± 1.79	1.58	1.77	3.29	0.32	0.24	1
	Control 2	6.06 ± 0.19	6.43 ± 0.37	5.4 ± 1.48	1	1	1	—	—	—
IPSV sol	*o*-Toluic acid	5.2	5.52	3.4	0.55	0.68	0.44	1	0.80	0.44
	m-Toluic acid	5.96	6.92	4.78	0.63	0.85	0.61	1.14	1	0.63
	p-Toluic acid	8.4	7.32	7.8	0.88	0.90	1	1.62	1.06	1
	Control 3	9.5	8.12	7.8	1	1	1	—	—	—

S_0: uptake of *o*-isomer to the *o*-isomer imprinted MIP. S_m and S_p for *m*- and *p*-isomer, respectively.
S: uptake of an isomer to a MIP.

μmol/g according to the isomer. It was only 3% of its maximum possible rebinding value. The reason could be that the recognition sites were placed on the surface of the MIP powder, and the sites inside the powder were mostly buried; moreover, the diffusion depth was thin because of the rigidity of the polymer in an aqueous solution. Uptakes for isomers other than the template isomer were much smaller than those of the control polymer. This was a clue to the recognition mechanism: the size and shape of the imprinted cavity controlled the rebinding. The rebinding sites were kept in the original structures of the imprinted cavities without distortion in IPSV because the extraction was conducted with a rigid powder. After IPSV was dissolved in toluene instead, its selective rebinding character completely disappeared. The reason was that the unique imprinted cavities were destroyed through the dissolution of the MIP polymer. However, the uptakes of dissolved MIPs slightly increased. There may have been a growth of pores in the MIP powder after its dissolution. Interestingly, the uptake of *p*-toluic acid was much lower than that of the other two isomers. *p*-Toluic acid has a much higher melting point (180°C) than the *o*-form (107°C) and *m*-form (111°C) and has the lowest solubility in water of the isomers according to the Merck Index. The high melting point of *p*-toluic acid implies that it is strongly associated as a dimer. During the imprinting process with vinyl pyridine, the dimer can be dissociated into a monomer form by acid–base interactions. Thus, the resulting MIP makes a cavity for a single molecule rather than a dimer form. The low solubility in water implies that the *p*-isomer may exist mostly as a dimer even in aqueous solutions. Because the dimer form needs more space to rebind, the uptake of *p*-isomer can be lower. For the controls and dissolved IPSV sol in toluene, the uptakes for all the isomers were close. This was due to the destruction of the unique cavity for the selectivity.

IPD was swollen in a mixed solvent of toluene and ethanol to remove the template. Then, it was made a powder (60 mesh). The uptakes were 18–47 μmol/g of MIP, depending on the guest molecule. It was about 17% of the calculated maximum. The amount was nearly 5 times higher than that of IPSV for the *m*-isomer. Because of the swelling, the powders had an increased number of rebinding cavities on the surface and pores in MIP particles to make the diffusion of guest molecules easier. In contrast to swollen IPD, IPD removed templates by the same method as IPSV, in a nonsolvent for the polymer but in a solvent for the templates, and was very similar to IPSV in both uptake and selectivity. The results are not shown here. However, for IPSV sol, which removed templates in a solution state with a mixed solvent, no specific selectivity was shown. These results imply that the swelling of MIPs in a certain range does not destroy the three-dimensional recognition cavities that control the selectivity.

An optimum crosslinker concentration was found to exist that did not sacrifice the unique character of MIPs during the swelling process for the removal of templates. For our experiments, the IPD composition in Table I was the best for the uptake and selectivity and for the ease of template removal in organic solvents.

CONCLUSIONS

MIPs with styrene and vinyl pyridine were prepared with and without DVB as the crosslinker. The MIPs without DVB showed high selectivity to the imprinted molecule but small uptakes. After they were dissolved in a mixed solvent of toluene and ethanol, the unique selectivity disappeared, just as for the control polymer, because of the destruction of the imprinted cavities. MIPs with a certain amount of the crosslinker had much higher uptakes without any sacrifice of their selectivity, even after swelling in the mixed solvent. The removal of the template was faster and easier in the solvent. This meant that the original imprinted cavities were maintained reversibly without any destruction of them by the crosslinker throughout the template extraction process in the swollen state. Thus, there are optimal ranges for the crosslinker concentration in the preparation of MIPs that maintain the selectivity with high uptake.

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