J Mater Sci (2009) 44:6206–6211
DOI 10.1007/s10853-009-3863-3

Preparation and characterization of molecularly imprinted polymers for the selective separation of 2,4-dichlorophenoxyacetic acid

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Received: 8 April 2009 / Accepted: 31 August 2009 / Published online: 10 September 2009

Abstract As a method of preparing ligand-selective cavities in a synthetic polymer matrix, molecular imprinting technique has been attracting significant interest from a large number of areas in chemistry and analytical sciences. In this study, molecularly imprinted polymers (MIPs) were prepared with styrene, 4-vinylpyridine (4-VPy), and divinylbenzene (DVB) for the separation of hazardous 2,4-dichlorophenoxyacetic acid (2,4-D), and the selectivity of MIPs as adsorbed 2,4-D and structurally similar materials was evaluated. The template was removed through the swelling process of toluene/ethanol, and the removal ratio was about 95–99%, respectively. MIPs synthesized in this study had good adsorption selectivity in the presence of other materials, although there was a difference of adsorption quantities (uptake) in the functional monomer (4-VPy contents) and the cross-linker (DVB contents). The results exhibit that the selectivity of MIPs was improved significantly by controlling the cross-linker. We expect that molecular imprinting technique will serve as a novel method for selective separation of specific materials in various fields, especially in the fields of environment and pharmaceutics.

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Introduction

As a method of preparing ligand-selective cavities in a synthetic polymer matrix [1–5], molecular imprinting technique has been focused on the possibility of creating molecular recognition sites that have high selectivity to the target molecule into polymeric materials. The technique has attracted significant interest from chemistry and analytical sciences owing to the relative ease with which these polymers may be prepared, to the range of template structures amenable to use, and to the apparent mechanical and chemical stability of the types of polymers generally synthesized [6]. The molecularly imprinted polymers (MIPs) are synthesized by the co-polymerization of functional vinyl monomers with a divinyl monomer as a cross-linker in the presence of template. After the removal of the template using appropriate solvents, the resulting polymers have a memory to selectively recognize the guest molecule using as a template through the footprint-like vacant spaces as a host.

MIPs are generally prepared by using the covalent bonding procedure proposed by Wulff [7] and non-covalent bonding procedure proposed by Mosbach [8]. By using the covalent bonding procedure, 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 non-covalent bonding procedure is very easy to carry out. In this method, a highly cross-linked polymer interacts with the template molecule simply through electrostatic interaction, hydrogen bonding, or similar non-covalent bonds. After the retained template is washed out, the polymer shows a formation of complementary sites with significant molecular recognition properties.

MIPs have been applied as affinity chromatographic stationary phase [9, 10], artificial antibodies [11, 12], synthesis mediators [13, 14], sensor components [15, 16], and adsorbents for solid phase extraction [17, 18].

In recent years, the increase in production and use of pesticides for agriculture has resulted in the presence of a variety of persistent contaminants in the environment, including in surface water and groundwater. Pesticide-related concerns in the world include water pollution due to runoff of the cultivated land, poor handling of pesticides in agricultural activities, little or no environmental monitoring, lack of safety measures and emergency plans, and disposal of pesticide containers without prior cleaning. Specifically, 2,4-dichlorophenoxyacetic acid (2,4-D) was selected in this study because it is an authorized pesticide and one of the most widely used. 2,4-D is a moderately toxic, inexpensive, and fairly selective herbicide. On account of its poor biodegradability, and as a consequence of its widespread use in agriculture, it can be detected as a pollutant in aquatic systems.

In this study, by using the molecular imprinting technique, we synthesized MIPs which can separate hazardous 2,4-D and confirmed the selectivity of MIPs by adsorbing materials similar to 2,4-D similar structure such as *o*-toluic acid (*o*-TA), *m*-toluic acid (*m*-TA), *p*-toluic acid (*p*-TA), benzoic acid (BA), aspirin (AS), 1-naphthol (1-NT), and 1-naphthoic acid (1-NA). The structure of each material is represented in Fig. 1.

Experimental

Materials

2,4-D, *o*-toluic acid (*o*-TA), *m*-toluic acid (*m*-TA), *p*-toluic acid (*p*-TA), aspirin (AS), 1-naphthol (1-NT) and 1-naphthoic acid (1-NA), styrene, 4-vinylpyridine (4-VPy), and divinylbenzene (DVB) were purchased from Aldrich Chemical Company, Inc. (Milwaukee, WI, USA), benzoic acid (BA) from Duksan (Pharmaceutical Co., Ltd, Korea), and α,α-azobis (isobutyronitrile) (AIBN), toluene, ethanol, and *n*-hexane from Junsei Chemical Co., Ltd (Tokyo, Japan). Styrene, 4-VPy, and DVB were distilled under vacuum to remove the inhibitors before polymerization.

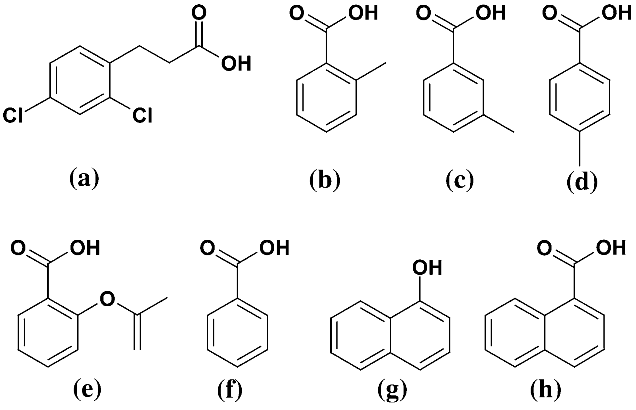

Fig. 1 Chemical structures of the adsorption materials used in this study. **a** 2,4-Dichlorophenoxyacetic acid (2,4-D); **b** *o*-Toluic acid (*o*-TA); **c** *m*-Toluic acid (*m*-TA); **d** *p*-Toluic acid (*p*-TA); **e** Aspirin (AS); **f** Benzoic acid (BA); **g** 1-Naphthol (1-NT); **h** 1-Naphthoic acid (1-NA)

Preparation of MIPs

2,4-D was mixed with 4-VPy to form a complex, and the complex was mixed with styrene, DVB as a cross-linker, and AIBN as an initiator. The mixing composition is shown in Table 1. The resulting solution was co-polymerized at 65 °C for 72 h under N_2 atmosphere. The control polymers are prepared without template for comparing the substrate uptake with the corresponding MIPs. The resultant polymers were ground in a mortar and passed through a 65 mesh (212 μm) sieve.

Removal and uptake of templates

The removal of template was carried out by using the method employed in our previous study [5]. The MIPs were swollen in toluene at first; then ethanol was added and stirred to extract the template effectively. The removal ratio of template was confirmed by UV–vis. spectrophotometer (UV-160A. Shimadzu, Kyoto, Japan).

The uptake was measured using UV–vis spectrophotometer after 20 mg of a dried MIP fine powder was added to 10 mL mixed solution (toluene:hexane) containing 6×10^{-6} mol of the guest molecules (2,4-D, *o*-TA, *m*-TA, *p*-TA, AS, BA, 1-NT, and 1-NA). A total of 20 mg of MIP contains 5.5×10^{-6} mol of template. The uptake of 2,4-D bound to the imprinted polymer uptake (mol/g), was calculated by the following Eq. 1:

$$\text{Uptake (mol/g)} = \frac{(C_0 - C_t)V}{W} \tag{1}$$

where C_0 and C_t are the concentration of 2,4-D, *o*-TA, *m*-TA, *p*-TA, AS, BA, 1-NT, and 1-NA (mol/L), measured at initial and post interval time (hour) for equilibrium. Symbols V and W are the volumes of the 2,4-D, *o*-TA, *m*-TA, *p*-TA, AS, BA, 1-NT, and 1-NA solution (10 mL), and the weight of dry polymer (20 mg) used for the adsorption experiment, respectively.

Selectivity factor (α) of the imprinted polymer is the relative value of 2,4-D, *o*-TA, *m*-TA, *p*-TA, AS, BA, 1-NT, and 1-NA bound to the imprinted polymer compared with that of 2,4-D template, and the selectivity factor was calculated by the following formula (2):

Table 1 The components of the reaction mixture for the preparation of the imprinted polymers [mol]

MIPs	Styrene	4-Vinylpyridine	Template	Divinylbenzene	AIBN (wt%)
DMIP1	30	1.0	1.0	1.0	0.1
DMIP2	30	1.5	1.0	1.0	0.1
DMIP3	30	2.0	1.0	1.0	0.1
DMIP4	30	2.5	1.0	1.0	0.1
DMIP5	30	3.0	1.0	1.0	0.1
DMIP6	30	1.0	1.0	1.5	0.1
DMIP7	30	1.0	1.0	2.0	0.1
DMIP8	30	1.0	1.0	2.5	0.1
DMIP9	30	1.0	1.0	3.0	0.1
Control1	30	1.0	–	1.0	0.1
Control2	30	1.5	–	1.0	0.1
Control3	30	2.0	–	1.0	0.1
Control4	30	2.5	–	1.0	0.1
Control5	30	3.0	–	1.0	0.1
Control6	30	1.0	–	1.5	0.1
Control7	30	1.0	–	2.0	0.1
Control8	30	1.0	–	2.5	0.1
Control9	30	1.0	–	3.0	0.1

$$\text{Selectivity factor } (\alpha) = \frac{\text{Uptake}_{(2,4-\text{D},o-\text{TA},m-\text{TA},p-\text{TA},\text{AS},\text{BA},1-\text{NT, and }1-\text{NA})}}{\text{Uptake}_{(2,4-\text{D})}} \quad (2)$$

where $\text{uptake}_{(2,4\text{-D},o\text{-TA},m\text{-TA},p\text{-TA},\text{AS},\text{BA},1\text{-NT, and }1\text{-NA})}$ was binding amounts of 2,4-D, *o*-TA, *m*-TA, *p*-TA, AS, BA, 1-NT, and 1-NA for DMIPs and $\text{uptake}_{(2,4\text{-D})}$ was binding amounts of 2,4-D for DMIPs.

Result and discussion

Removal of the template from MIPs

Molecular imprinting is a synthetic approach by which a molecular receptor is assembled by template-guided synthesis. Generally, molecular imprinting has largely been associated with polymer materials, e.g., MIPs. In the process of molecular imprinting, adequate functional monomers are introduced to interact with a template molecule, and the functional groups on the monomers are then fixed by chemical cross-linking of these monomers [19].

In general, most researches on the MIPs have used a very big portion of cross-linking monomer to maintain the three dimensional shapes for the template. Because the high concentration of cross-linking monomers was added, MIP was very rigid. The perfect removal of the template is very difficult owing to the formation of a rigid polymer network structure. The number of molecular recognition site becomes much smaller compared to the sites expected from the amount of the template used, because some binding sites are buried in the three dimensional network structure of polymers. To date, the removal of the template of MIPs has been carried out by using a simple washing or soxhlet extraction method because this method is easy to extract the template from the obtained polymer network. Nevertheless, the adsorption amount, i.e., the uptake, was relatively low. In this study, therefore, the third vinyl monomer (styrene) was used for preparing MIPs. Then, a small portion of the cross-linking monomer (DVB) was used in performance of the swelling process method [20] so that the adsorption will take place not just on the surface but also inside the polymer. Figure 2a shows the removal profiles on the various mixing ratios of toluene and ethanol. After swelling in toluene, ethanol was added to remove the template. From the swollen MIPs, templates can be dissolved more easily in a solvent than in the rigid state because of the increase of freedom in their diffusion. However, since ethanol is not a solvent to the polymer, the swollen MIPs in toluene was shrunk in proportion to the ethanol added. The higher the ethanol content, the more the polymer was shrunk. The optimum solvent mixing ratio was found when the mixture of toluene:ethanol = 2:1 (v/v) was used. Various MIPs were synthesized using 2,4-D as template by varying the quantity of the functional monomer (4-VPy) and the cross-linker (DVB). The removal ratio of the template with the extraction time is shown in Fig. 2b. The removal of template got easier as the amount

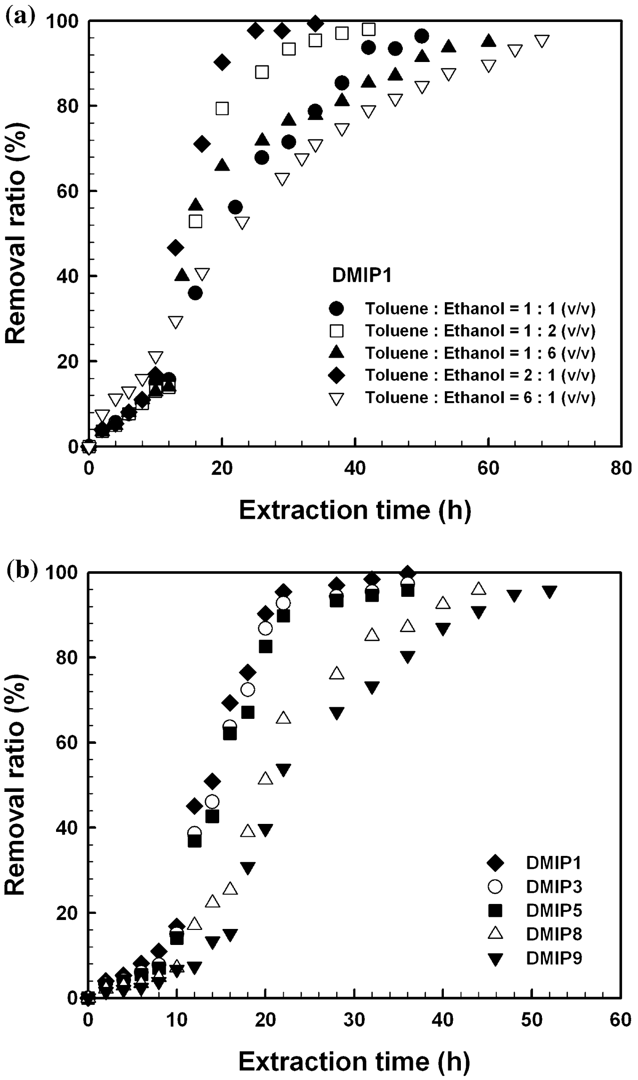

Fig. 2 The removal ratio of 2,4-dichlorophenoxyacetic acid (2,4-D) as the template. **a** The removal ratio of 2,4-D depending on various mixing ratio of toluene:ethanol (v/v). **b** The removal ratio of 2,4-D with the amount of DVB as cross-linker in a mixed solvent of toluene:ethanol = 2:1 (v/v)

of DVB became smaller. However, the removal ratio of template using the functional monomer of 4-VPy was similar to that of DMIP1.

The evaluation of MIPs

In order to identify the adsorption equilibrium and adsorption solution, we carried out an adsorption experiment which was adsorbed on MIPs (DMIP1) removed the 2,4-D as template with the time and mixed solvent ratio as adsorption solution (Fig. 3). As shown in Fig. 3, the adsorption rate reached the equilibrium after 3 h in each mixed solvent ratio. When the mixed solvent was used as adsorption solution, the highest value was found with the mixture of toluene:hexane = 1:4 (v/v).

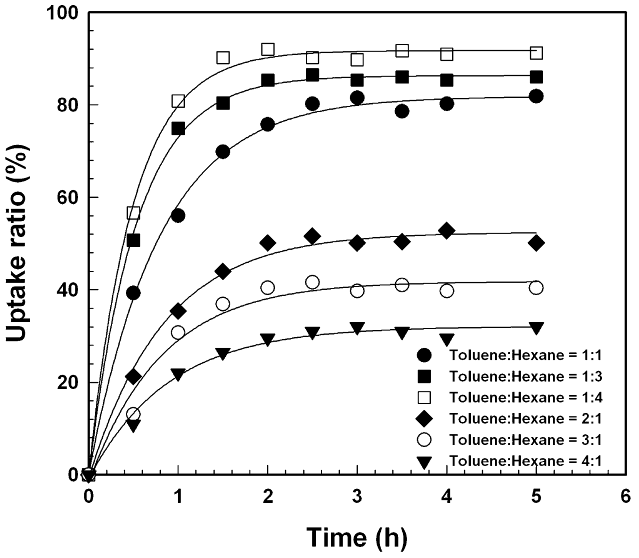

Fig. 3 The uptake ratio of the DMIP1 in terms of mixing ratio of toluene:hexane (v/v)

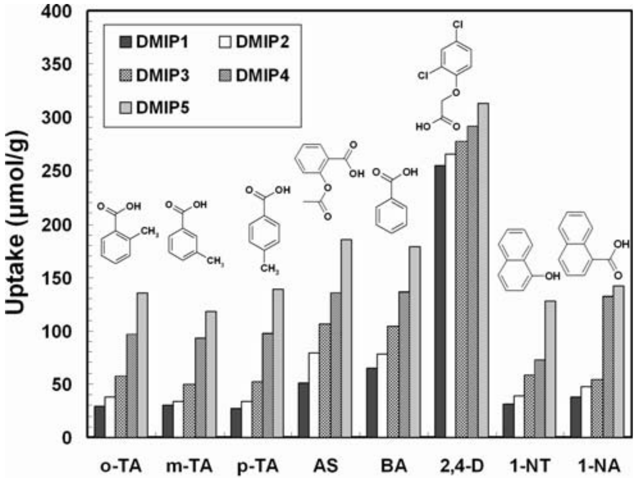

Fig. 4 Uptakes for DMIPs in term of 4-vinylpyridine (4-VPy) content as functional monomer

Figures 4 and 5 show the adsorption amounts of 2,4-DMIPs synthesized with different amounts of 4-VPy and DVB. All the DMIPs with the different mole ratio of 4-VPy and DVB showed selective separation. In the case of DMIPs (DMIP1, DMIP2, DMIP3, DMIP4, and DMIP5) prepared at the different mole ratio of 4-VPy (Fig. 4), the uptake was increased overall with increasing 4-VPy contents because adsorption occurred not only at the molecular recognition site formed by imprinted polymer network but also at the sites formed by increasing 4-VPy as a functional monomer. Figure 5 shows the uptake changes of DMIPs (DMIP1, DMIP6, DMIP7, DMIP8, and DMIP9) which were synthesized by the different mole ratio of DVB. All the 2,4-D imprinted MIPs show the highest uptake on

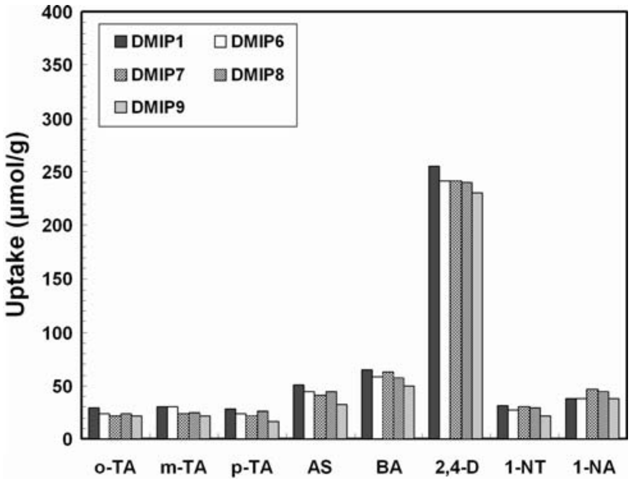

Fig. 5 Uptakes for DMIPs in term of divinylbenzene (DVB) content as cross-linker

2,4-D among the other materials regardless of the cross-linker amount, inducing from the general character of MIP. With increasing DVB contents, the uptake was decreased because MIP is more rigid at the higher content of DVB, and the rigidity makes it difficult to diffuse guest molecules into the MIP. However, the rigid MIPs may have higher selectivity to the imprinted material than to other materials because the rigidity can maintain the original imprinted cavity. The best MIP must have both high amounts of uptake and selectivity.

Figure 6a, b shows the uptake ratio of DMIPs for the uptake adsorbed in each adsorption materials to control polymers that are polymerized without the template. These values display the ability of MIPs to compare control polymers. These results indicate that the uptake ratio for 2,4-D is considerably higher than the others, also verify that the capability of the DMIPs synthesized by molecular imprinting technique is excellent.

Table 2 shows the selectivity factor (α) of DMIPs. The α value calculated by Eq. 2 expresses the degree of selectivity on prepared DMIPs. For example, when 2,4-D was adsorbed by MIP synthesized using 2,4-D as template, the α value is 1. However, the α value was smaller than 1 when the other materials were adsorbed. Having the α value lower than 1 implies that the selectivity was low for polymer imprinted by the specific molecule. From the result shown at Table 2, we observed that the α values of each DMIPs that were synthesized using 2,4-D as the template had a value smaller than 1 when *o*-TA, *m*-TA, *p*-TA, AS, BA, 1-NT, and 1-NA were adsorbed on DMIPs synthesized using 2,4-D. Also, a comparison of α values on the 4-VPy contents (DMIP1, DMIP2, DMIP3, DMIP4, and DMIP5) and the DVB contents (DMIP1, DMIP6, DMIP7, DMIP8, and DMIP9) indicated that the α values with the DVB contents were lower than the 4-VPy contents. With

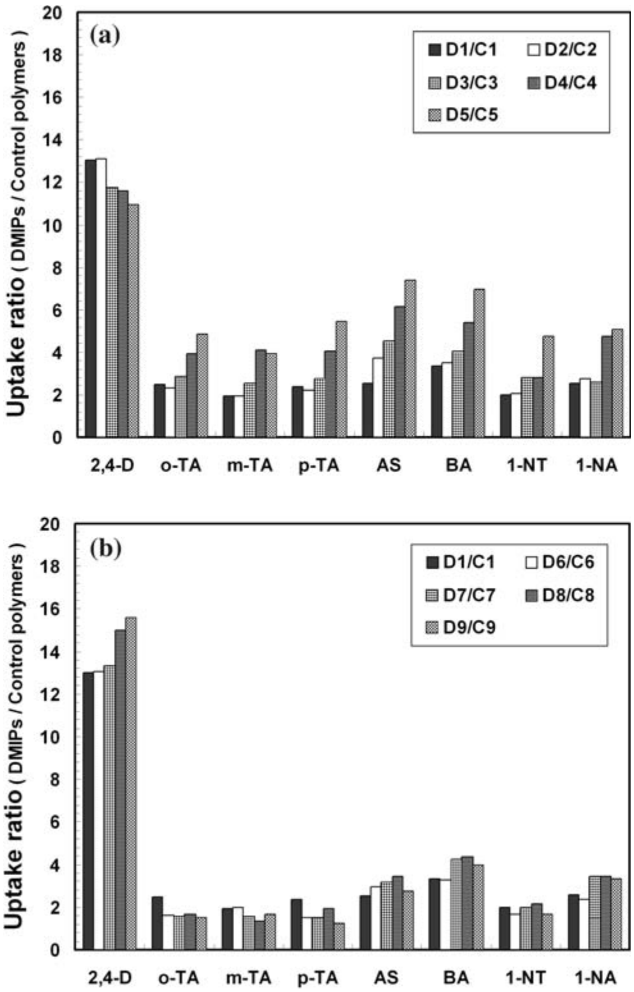

Fig. 6 The uptake ratio of various MIPs to the corresponding control polymers. **a** The uptake ratio of DMIPs to the corresponding control polymers with 4-vinylpyridine (4-VPy) content as functional monomer. **b** The uptake ratio of DMIPs to the corresponding control polymers with divinylbenzene (DVB) content as cross-linker. Where D1–D9 are the uptakes adsorbed each adsorption materials on DMIP1–DMIP9, and C1–C9 are the uptakes adsorbed each adsorption materials on Control1–Control9

Table 2 Selectivity factor (α) of various MIPs

MIPs	Templates							
	2,4-D	*o*-TA	*m*-TA	*p*-TA	AS	BA	1-NT	1-NA
DMIP1	1	0.115	0.119	0.108	0.199	0.253	0.121	0.149
DMIP2	1	0.144	0.128	0.125	0.297	0.293	0.146	0.178
DMIP3	1	0.208	0.179	0.186	0.382	0.382	0.211	0.197
DMIP4	1	0.332	0.321	0.335	0.462	0.462	0.251	0.454
DMIP5	1	0.433	0.376	0.442	0.594	0.594	0.409	0.455
DMIP6	1	0.101	0.123	0.101	0.182	0.243	0.111	0.159
DMIP7	1	0.092	0.103	0.089	0.175	0.263	0.13	0.198
DMIP8	1	0.105	0.095	0.111	0.192	0.244	0.126	0.188
DMIP9	1	0.092	0.101	0.071	0.142	0.216	0.096	0.166

increasing DVB contents, the degree of selectivity of DMIPs was increased because the imprinted cavity was shaped very well as DVB as cross-linker increased. These findings led us to conclude that the three dimensional structure of MIPs was formed well, and highly selective MIPs were prepared when a small amount of DVB was increased.

Conclusion

Polymers capable of separating 2,4-D which can be used for herbicide were synthesized by applying the molecular imprinting technique. The removal of the template (2,4-D) was carried out using a mixed solvent (toluene/ethanol) in the swelling process. The adsorption ability of MIPs was estimated by using the adsorbed 2,4-D and materials structurally similar to 2,4-D. Also, it was evaluated by the selectivity factor (α). As the result of the adsorption ability of MIPs preparing with the changes in 4-VPy contents as functional monomer and DVB contents as cross-linker, with the increase of 4-VPy contents, the uptake was increased because adsorption took place not only in the imprinted space but at all adsorption sites with the increase of functional groups. For this reason, the selectivity for the separation of the template was decreased. With the increase of DVB contents, the uptake was decreased whereas the selectivity was increased because MIPs with the higher DVB content are more rigid. The increase of the rigidity of MIPs makes it difficult to diffuse guest molecules into the MIPs. On the other hand, the rigid MIPs could have higher selectivity to the imprinted molecule than those to others because the rigidity can maintain the original imprinted cavity. Compared to the control polymers which did not imprint the template, the adsorption selectivity of DMIPs was high. These findings led us to believe that the molecular imprinting technique can serve as a new method of separating various phenolic, chiral, and pharmaceutic compounds.

Acknowledgements This work was supported by the Korea Research Foundation Grant funded by the Korean Government (MOEHRD) (KRF-2007-412-J02001).

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