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Retention behavior of phenoxyacetic herbicides on a molecularly imprinted polymer with phenoxyacetic acid as a dummy template molecule

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Abstract—Molecular imprinted polymers (MIPs) binding with phenoxyacetic acid (PA) as a dummy template molecule were synthesized via thermal initiation in aqueous medium. The retention behaviors of benzoic acid (BA), PA, 2-methyl-4-chlorophenoxyacetic acid (MCPA), 4-chlorophenoxyacetic acid (4-CPA), and 2,4-dichlorophenoxyacetic acid (2,4-D) on this MIP column indicate that this material can selectively retain phenoxyacetic herbicides. To investigate these recognition mechanisms, the interactions between the functional monomer 4-vinylpyridine (4-VP) and PA or 2,4-D were investigated by computational modeling. ^{1}H NMR spectroscopy of 2,4-D titrated by 4-VP was recorded. The chemical shift of the 2,4-D acidic proton (12.15–14.32 ppm) shows the existence of the ion-pair interaction. This kind of polymers could be useful as stationary phases to extract 2,4-D, 4-CPA or MCPA and avoid leakage of a trace amount of target analyte remaining in the MIPs.

1. Introduction

Molecularly imprinting utilizing synthetic material to mimic natural receptors as a recognition element to provide selectivity and sensitivity against target molecules for affinity-based separations, biomimetic receptors, and sensors is becoming an important technique.[1–3] Molecularly imprinted polymers (MIPs) are polymerized by crosslinking functional monomer(s) onto polymer networks in the presence of a template molecule. Removal of the template reveals binding cavities fitted to the template. Functional monomers situated in the cavity lead the template to selectively rebind into the cavity again.

Phenoxyacetic acids are a class of important plant growth regulators and herbicides widely used in agriculture. Over the past eight years, MIPs for phenoxyacetic acid herbicides have proliferated to resolve the difficulties of detecting these compounds in the environment. Their applications cover artificial antibody,[4,5] solid phase,[6–11] and recognition elements of sensors.[12–17]

However, because MIPs are prepared by using the target analyte as template molecule, the leakage of a trace amount of the imprinted molecules remaining in the MIPs has hindered the accurate and precise assay of the target analyte.[18] A strategy to solve this problem is the utilization of an analyte analog (dummy template), instead of the analyte itself, as template, during polymerization.[19,20] In the study described here, the flavoring and intermediate (for manufacturing dyes, pharmaceuticals, pesticides, fungicides) phenoxyacetic acid (PA) were chosen to prepare dummy molecularly imprinted polymer (DMIP) to selectively retain phenoxyacetic herbicides.

Figure 1 shows the process of imprinting. On these polymers, the retention behaviors of dummy template PA, control analog BA, and phenoxyacetic herbicides (MCPA, 4-CPA, and 2,4-D) were investigated. BA molecule absents the oxygen bridge sub-structure comparing with template molecule. Phenoxyacetic herbicides differ with template of the aromatic part. Strong acidic aqueous mobile phase was used, to facilitate ion-pair and hydrophobic interaction, shorten the analytical time,

Keywords: Molecularly imprinted polymers; Phenoxyacetic herbicides; Phenoxyacetic acid; Molecular modeling.
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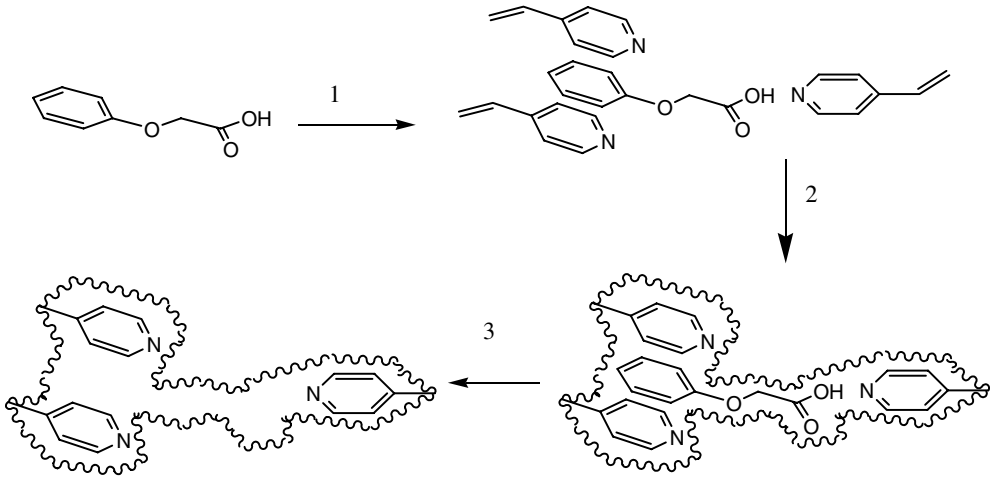


Figure 1. The process of imprinting PA. 1 = Self-assembly of the template and functional monomer; 2 = incorporation of the functional monomer–template complex into the polymer matrix; 3 = removal of the template molecule and formation of the cavity.

and avoid tailing peaks. As the elution strength of water may affect the binding of MIP with template, it is seldom used as the mobile phase in HPLC evaluation.

Computational molecular modeling and ^{1}H NMR were employed to investigate the natural affinity between PA or 2,4-D and 4-VP. A clear understanding of the recognition mechanism governing the selectivity of MIPs allows further application of this material. Computational design and modeling method enabled the Piletsky and Tunon-Blanco group to select, from a virtual library, high affinity functional monomers interacting strongly with the target analyte.[21–23] In the present paper, the natural affinity was explained by computational molecular modeling simulating the interaction between 4-VP and 2,4-D as compared to that of PA. The binding energies of the complexes, bond lengths, and Mulliken atomic charges were computed by a workstation Gaussian 03 program package.[24]

In a previous study, the application of ^{1}H NMR spectroscopy as a tool for probing the complexation process in the prepolymerization mixture was discussed.[10,25–27] For the hydroxyl proton, vinylpyridine (VP) prefers to provide stronger points of recognition than methacrylic acid (MAA).[25] Mizaikoff's group investigated the 2,4-D and 4-VP complex by titrating pyridine-d_5 to 2,4-D in D_2O and proposed the conformation of formed 3:1 4-VP:2,4-D complex.[10] In this study, ^{1}H NMR spectra of 2,4-D/4-VP at ratios between 4 + 1 and 1 + 4 were recorded in $CDCl_3$ to show existence of the ion-pair interaction. The formation of a sufficiently strong complex between 2,4-D and 4-VP is critical to the subsequent recognition properties of the polymer. The chemical shift changes of acid proton and pyridyl proton are discussed here.

2. Results and discussion

2.1. Chromatographic experiments

As a typical target molecule, strong acid herbicide 2,4-D was imprinted several times in the presence of the polar solvents methanol and water, as proposed by Haupt in 1998.[4,5] In Haupt's work, structures of different compounds such as 2,4-dichlorophenoxybutyric acid (2,4-DB) and 4-chlorophenoxyacetic acid (4-CPA) were used as competitors in radioligand binding assays. The corresponding cross-reactivities were 95% and 24% (that of template 2,4-D was 100%) relating to the match between competitor and cavity.[4,5] This principle was also applied to a competitive electrochemical assay or sensor format.[12] As a much stronger acidic template, 2,4,5-T was imprinted also, and the MIPs were tested as solid-phase extraction (SPE) sorbent and as a chromatographic stationary phase. The selectivity of the MIPs for the template was overwhelming.[6–9]

However, a trace amount of the imprinted molecules remains in the MIPs even if the MIPs had been extensively washed.[20] The bleeding template phenomenon could result an inaccurate quantification, especially at residual level in SPE. Dummy template can be used to avoid this problem. Even if the dummy template bleeds from MIPs, it can be separated from target molecules in the chromatographic analysis followed. DMIP should selectively retain target molecules and separate impurity with target analyte. In this work, PA was used as the dummy templates to prepare MIPs to retain phenoxyacetic herbicides MCPA, 4-CPA, and 2,4-D.

The retention behavior of phenoxyacetic herbicides on MIPs was investigated by HPLC. The relative performance of MIPs or blank polymers is gauged by its ability to selectively retain the template or analog. The capacity factors (k') of the prepared MIPs are evaluated as: $k' = (t - t_{\text{methanol}})/t_{\text{methanol}}$, where t is the retention time of the analyte and t_{methanol} is the retention time of the void marker methanol (MeOH) on the column.[28]

The chromatograms of PA, BA, MCPA, 4-CPA, and 2,4-D on imprinted polymer and the blank polymer column are shown in Figure 2. The capacity factors of PA, BA, MCPA, 4-CPA, and 2,4-D on MIPs are 0.995, 0.505, 1.266, 1.599, and 2.147 (Fig. 2a), while

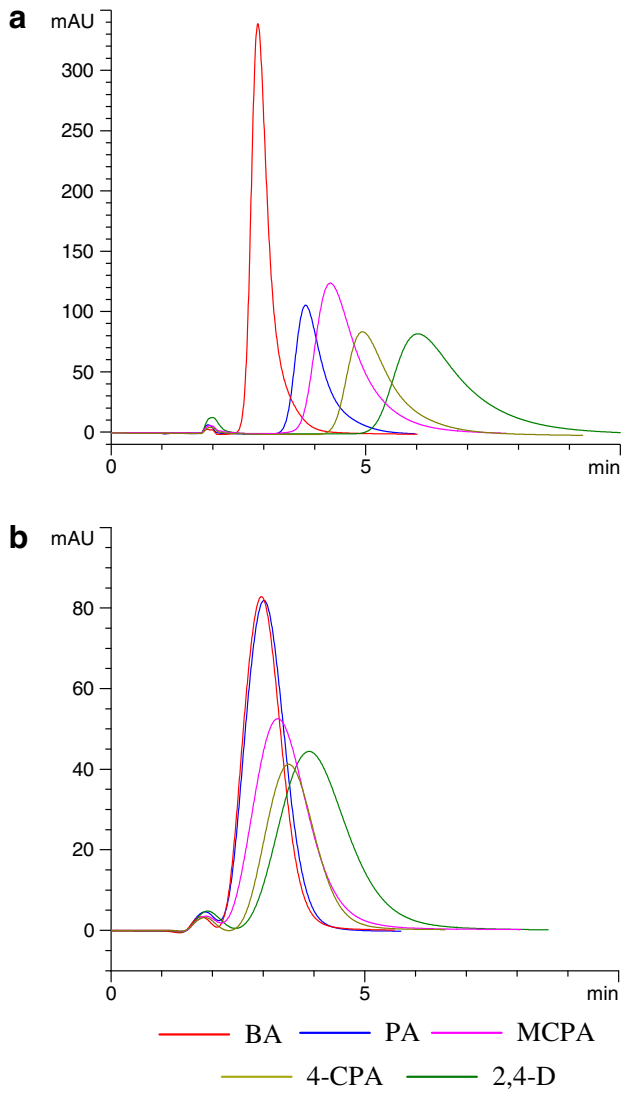


Figure 2. Chromatograms of BA, PA, MCPA, 4-CPA, and 2,4-D on imprinted polymer column (a) and the blank polymer column (b). Mobile phase: acetonitrile/water/acetic acid (93 + 5 + 2, v/v); flow rate: 1.0 mL min^{-1}.

those on blank polymers are 0.530, 0.504, 0.729, 0.843 and 0.982 (Fig. 2b), respectively. The retention results demonstrate that because of the imprinting effect, PA matching the cavity is recognized and retained more than BA. Without the oxygen bridge sub-structure, BA, whose phenyl is too big to fit into the narrow part of the cavity matching the oxygen bridge sub-structure, shows poor retention on MIPs due to the weakened interaction between the carboxyl and the basic nitrogen of the pyridyl. The retention of BA also improves because of the hydrophobic interaction, such as the π–π stacking effect, explained in previous work.[4,10] When the aromatic part of PA was imprinted, two pyridyls and a phenyl of PA form a sandwich structure (Fig. 1). The aromatic portion of BA is the same as that of PA. Because of this π–π stacking structure, BA can embed into this cavity. But, compared with PA, which reacts with the binding site not only by π–π stacking but also by ion-pair interactions, BA's improvement is trivial. The evidence above indicates that ion-pair interaction is the most important factor for selectivity of MIPs.

Generally, DMIP is inferior to the MIPs in terms of selectivity for the target analyte.[18] However, the phenomenon that the affinity of the analog is higher than that of the template on template MIPs was reported previously.[22] In this study, the retention time of MCPA, 4-CPA or 2,4-D on PA MIP column was longer than that of PA. The retention behavior of the analyte is affected by ion-pair interaction between the carboxyl and the basic nitrogen of the binding site, hydrophobic interaction between pyridyl and phenyl, and shape complementarity between the analyte molecule and the binding cavity. The oxygen bridge and carboxylic sub-structure of MCPA, 4-CPA or 2,4-D is similar to that of template PA, which allows the oxygen bridge and the carboxyl to pass through the narrow part of the cavity and react with basic nitrogen inside the cavity. The fact discussed above shows that the hydrophobic interaction is too weak to bind the aromatic part of PA. The MIPs were synthesized via thermal initiation, which induces the aromatic part to vibrate and imprint incorrectly. The cavity neglects the shape difference between PA and MCPA, 4-CPA or 2,4-D. So, the strength of ion-pair interaction is the most important factor to effect retention behavior.

2.2. Computational modeling and NMR spectroscopy

Molecular modeling has been used to predict which functional monomers are capable of forming effective polymers.[21,22] To investigate the natural affinity of 2,4-D to PA MIPs, a simulation of interaction between 4-VP and PA or 2,4-D is adopted here. Optimized geometries for the most stable complexes are shown in Figure 3. Considering the main force of 2,4-D combining to MIPs is ion-pair, the energies of one PA molecule (or one 2,4-D molecule) interacting with one 4-VP molecule are calculated.

Table 1 shows the simulative binding energy values of the 1:1 PA (or 2,4-D):4-VP complex. The fact that the lower binding energy leads to the more stable complex implies that 2,4-D has a decisive advantage to combine to MIPs compared to PA. The binding energy (ΔE) is calculated using this equation:

$$\Delta E = E(\text{template} - \text{monomer}) - [E(\text{template}) + E(\text{monomer})]$$

The binding energies of 4-VP and 2,4-D or AP listed in Table 2 indicate that 2,4-D has a natural affinity to 4-VP. This fact also can be observed from Figure 2b showing the retention time of 2,4-D is longer than that of PA on blank polymer.

^{1}H NMR spectroscopy can provide useful information on the study of the intermolecular interactions between the template and functional monomers. Chemical shift migration behavior in different solvents can determine the recognition mechanism of the imprinted

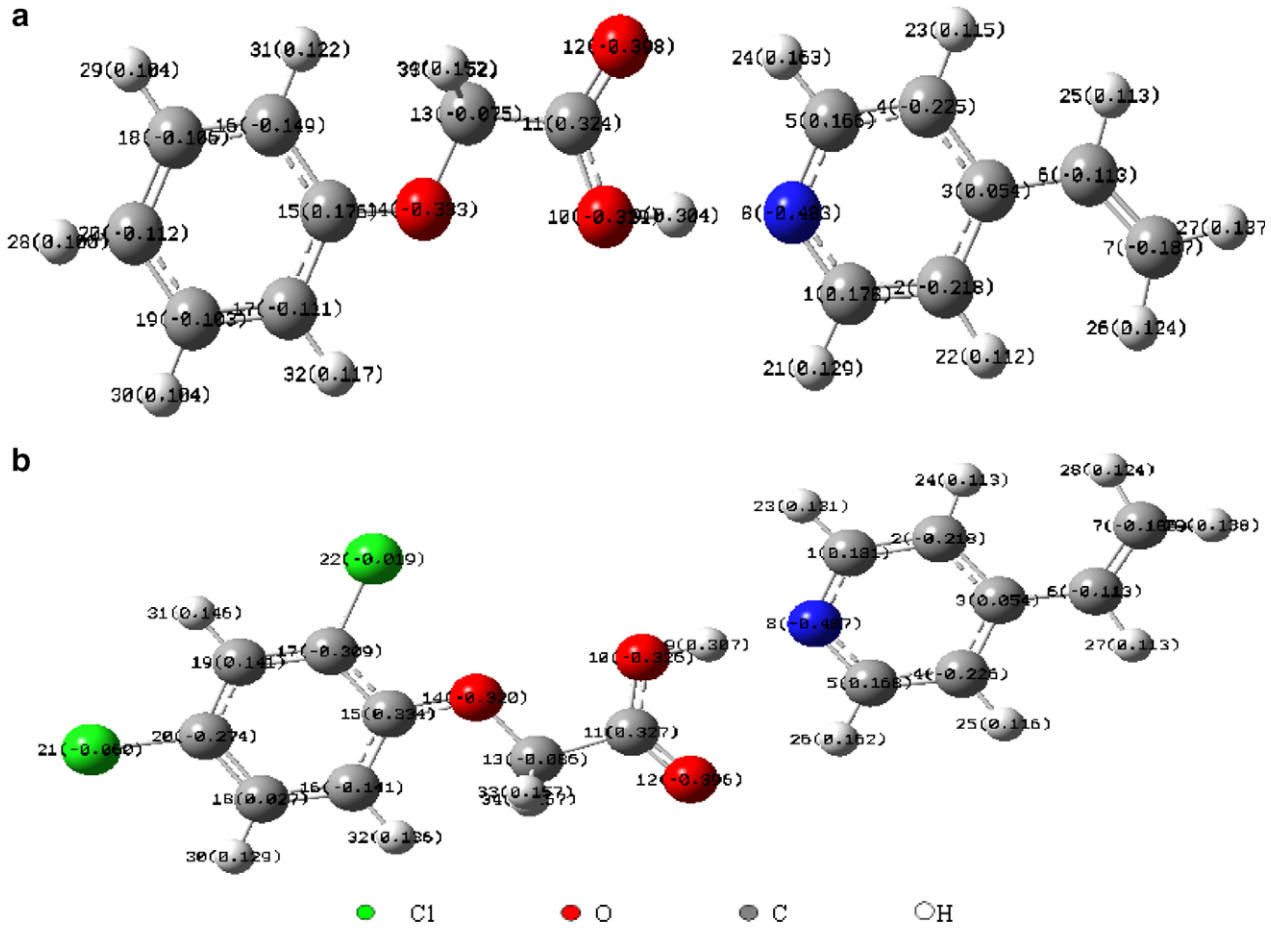


Figure 3. Optimized geometries for the most stable complexes of PA (a) and 2,4-D (b) with 4-VP separately. Mulliken atomic charge Q in e.

Table 1. Computational modeling data: bond length r (Å), Mulliken atomic charge Q (e), and binding energy ΔE (kcal mol^{-1})

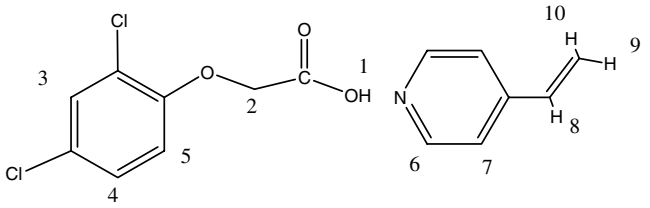
	$r^{c}_{\text{O–H}}$ (Å)	$r_{\text{H}\cdots\text{N}}$ (Å)	$Q_{\text{O}}{}^{d}$ (e)	Q_{H} (e)	Binding energy ΔE (kcal mol^{-1}) in vacuum
PA	0.967	—	−0.294	0.256	—
2,4-D	0.967	—	−0.290	0.260	—
Complex[a]	1.013	1.666	−0.329	0.304	−14.066
Complex[b]	1.017	1.656	−0.326	0.307	−14.723

[a] Complex: 1:1 PA:4-VP complex.
[b] Complex: 1:1 2,4-D:4-VP complex.
[c] r: bond length.
[d] Q: Mulliken atomic charge.

polymers because the recognition of imprinted polymers is based on these interactions. As reported, ^{1}H NMR spectra of template were recorded with acidic or basic monomers in various polar media.[26,27] The interaction between 2,4-D and 4-VP was studied in previous work by recording ^{1}H NMR spectra of 2,4-D at constant concentration titrated by monomer at increasing concentration in $CDCl_3$ and D_2O. However, pyridine-d_5 was used instead of 4-VP to facilitate the observation of aromatic proton shifts. The chemical shift migration of aromatic protons on 2,4-D indicates the conformation of the formed 3:1 4-VP:2,4-D complex in aqueous solvent. One 4-VP molecule attracts a 2,4-D molecule by electrostatic interaction, two 4-VP molecules sandwich one 2,4-D molecule by hydrophobic interaction, like the structure of the complex in Figure 1. Without an aqueous environment inducing hydrophobic phenomenon, only an ionic interaction is observed in $CDCl_3$.[10] According to the chromatographic result shown above, the ion-pair effect is more important than the hydrophobic effect even in aqueous mobile phase. Therefore, the ion-pair effect was investigated in $CDCl_3$.

In this work, to record the pyridyl proton spectrum, 4-VP was used to titrate 2,4-D. Table 2 shows the chemical shifts of protons about 2,4-D titrated by 4-VP at different ratios in $CDCl_3$, and the ratios of 2,4-D/4-VP are 4:0, 4:1, 2:1, 1:1, 1:4, and 0:4. The dramatic downfield chemical shift of the carboxyl proton suggests that the ion-pair interaction between 2,4-D and 4-VP is significant. The evidence that the chemical shifts of the pyridyl protons increased after interaction with 2,4-D proves that 4-VP provides

Table 2. The proton chemical shift (ppm) of 2,4-D titrated by 4-VP



	Chemical shift (ppm)									
	H1[d]	H2	H3	H4	H5	H6	H7	H8	H9	H10
2,4-D[a]	9.7000	4.7429	7.4051	7.1829	6.8006	—	—	—	—	—
4:1[b]	12.1521	4.6981	7.3802	7.1538	6.8152	8.7122	7.5714	6.7588	6.1700	5.7784
2:1	13.3973	4.6920	7.3804	7.1459	6.8231	8.6630	7.5020	6.7360	6.1158	5.7006
1:1	13.9430	4.6951	7.3688	7.1441	6.8400	8.6053	7.3916	6.7037	6.0506	5.6080
4:4	14.3268	4.7043	7.3336	7.1442	6.8536	8.5704	7.2921	6.6660	5.9817	5.5094
4-VP[c]	—	—	—	—	—	8.5640	7.2773	6.6168	5.9728	5.4905

[a] 2,4-D: 2,4-D in $CDCl_3$ without 4-VP.
[b] 4:1: 2,4-D:4-VP.
[c] 4-VP: 4-VP in $CDCl_3$ without 2,4-D.
[d] H: proton position marked aloft.

electrons to 2,4-D when forming the ion-pair complex. The increasing 4-VP concentration brings the signal acutely downfield. Because the hydroxyl oxygen atom of 2,4-D magnetizes the electron of the acidic proton to ionize when a nitrogen atom approaches, the chemical shift of the acidic proton moves downfield. Although the insufficiencies of the solvent polarity, the 4-VP quantity, and the distance between the nitrogen atom and proton inhibit the ionization of 2,4-D and 4-VP, a strong ion-pair interaction enables them forming stable complex. Chemical shifts of methylene and aromatic protons change slightly, which proves that the hydrophobic interaction in $CDCl_3$ is secondary. Therefore the interaction between 2,4-D and 4-VP is mainly due to an ion-pair interaction.

The molecular modeling supports this assumption appropriately (Table 1). According to the molecular modeling, the acidic proton Mulliken atomic charge of 2,4-D is 0.260 e in vacuum, while that of 1:1 2,4-D:4-VP complex is 0.307 e. The positive atomic charge increases due to the deshielding effect of protonation by the oxygen atom whose negative charge increases from 0.290 to 0.326 e indicating the downfield shift of the carboxyl proton. The simulative bond length between carboxyl oxygen and proton increases from 0.97 to 1.02 Å after combining with pyridine, while the bond length between the nitrogen atom and the proton is 1.656 Å in this simulative vacuum condition. Given that a general single bond length of oxygen and proton is 0.97 Å, and that of hydrogen bond is 1.80 Å, the interaction between 2,4-D and pyridine is ion-pair interaction stronger than general hydrogen bond.

3. Conclusion

Molecular imprinted polymers (MIPs) binding with PA as a dummy template molecule can selectively retain phenoxyacetic herbicides. This kind of polymers could be useful as stationary phases to extract and concentrate phenoxyacetic acid herbicides and avoid leakage of a trace amount of the imprinted molecule remaining in the MIPs. To investigate these recognition mechanisms, the interactions between the functional monomer 4-vinylpyridine and PA or 2,4-D were investigated by computational modeling. The chromatographic behavior, NMR spectroscopy, and molecular modeling data demonstrate that the strength of the ion-pair interaction is the most important force for molecular recognition. The hydrophobic effect is trivial.

4. Experimental

4.1. Chemicals

4-CPA(99%), MCPA(95%), 2,4-D(95%), and PA(99%) were supplied by Pesticide Analysis Laboratory China Agricultural University (Beijing, China), 4-VP, ethylene glycol dimethacrylate (EGDMA), and chromatograph solvents were purchased from J&K Chemical Ltd (Beijing, China), all other reagents were from Beijing Chemical Reagents Company (Beijing, China). Purified water was from Wahaha Group Co., Ltd (Beijing, China). $CDCl_3$ + 0.03%(v/v) tetramethyl silane (TMS) used for NMR analysis were from Cambridge Isotope Laboratories, Inc., USA.

4-VP was distilled at reduced pressure and stored in the dark at −18 °C. EGDMA was purified by basic alumina column immediately before use.

4.2. Polymer synthesis

In a 20-mL glass ampoule, a solution was prepared by dissolving 1 mmol of template and 4 mmol of monomer (0.425 mL of 4-VP) into 1 mL of methanol and 1 mL of water. The solution was placed in a refrigerator at 4 °C

overnight to allow the template to incubate with 4-VP. Then, 20 mmol of crosslinker (3.765 mL of ethylene glycol dimethacrylate), 0.31 mmol of initiator (0.051 g of 2,2′-azobisisobutyronitrile), and 3 mL of methanol were dissolved in this solution. The mixtures were sonicated, sparged with nitrogen for 5 min, sealed, and placed in a water bath at 45 °C for 4 h, followed by 20 h at 60 °C. The blank polymer was synthesized in the same method, but without template. The polymers obtained were removed from the glass ampoules, ground in an electric mixer mill (MM301 Retsch) for 3 min, and wet-sieved to 20–80 μm in size.

The obtained particles were suspended in ethanol/water (50 + 50, v/v) and mechanically slurry packed into HPLC stainless steel columns (length, 15.0 cm, id 4.6 mm) with methanol at constant pressure of 30 MPa.

4.3. Chromatographic experiments

In order to remove the template, the columns were washed with acetonitrile/acetic acid (90 + 10, v/v) for 2 h, then by the mobile phase consisting of acetonitrile/water/acetic acid (93 + 5 + 2, v/v). HPLC analyses were performed on an Agilent Model 1100 Series LC using the following operating parameters; flow rate: 1.0 mL min^{-1}; temperature: 25 °C; injection volume: 10 μL; Diode Array Detection at the following wavelengths: 270 nm for PA and BA, 285 nm for 4-CPA, MCPA and 2,4-D. The standard solutions were prepared by dissolving 10 mg of MCPA, 4-CPA, 2,4-D, PA, or BA into 10 mL of methanol to obtain concentrations of 1.0 g L^{-1}.

4.4. Molecular modeling

All calculations included in this work were performed with the Gaussian 03 program package. The geometry of each structure was fully optimized by using the density functional method (pbe1pbe) with 6-311g (d,p) basis set. All the geometric parameters of the possible stationary points were located and characterized by the number of imaginary frequencies. All the energies of the structures have zero-point energy (ZPE) correction.

4.5. NMR spectroscopy

^{1}H NMR spectra were recorded on a Bruker DPX 300 MHz NMR spectrometer (Bruker Biospin, UK) at 25 °C. The samples were prepared by dissolving 0.05 mmol 2,4-D with 0.0125, 0.025, 0.05 or 0.2 mmol 4-VP, respectively, in 500 μL $CDCl_3$ (TMS as internal standard) and stored at −18 °C overnight to stabilize the complex.

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