Molecularly Imprinted Polymer Membranes for Substance-Selective Solid-Phase Extraction from Aqueous Solutions

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ABSTRACT: Thin-layer molecularly imprinted polymer (MIP) composite membranes for selective binding of monocrotophos (MCP) pesticide from aqueous solutions were developed. The procedure was based on commercially available membrane modules that were rinsed with prepolymerization imprinting mixtures. After the *in situ* polymerization and generation of MIP films on the membranes within the modules, the membranes were evaluated in terms of affinity toward the target molecule MCP. MIP membranes with different porogens and different monomers on Nylon-6 membranes were prepared. It was shown that MIP membranes synthesized with methacrylic acid as monomer and toluene as porogens on the Nylon-6 membranes provided a highly selective binding of MCP from aqueous solutions under the optimized elution conditions. With the novel surface modification technique, the low nonspecific binding properties of the microfiltration membrane could successfully be combined with the receptor properties of molecular imprints, yielding substance-specific MIP composite membranes. The high affinity of these synthetic membranes to MCP pesticide together with their straightforward and inexpensive preparation could be applied in a fast preconcertration step, solid-phase extraction, by a simple microfitration for the determination of MCP in water. © 2006 Wiley Periodicals, Inc. J Appl Polym Sci 101: 4468–4473, 2006

Key words: molecular imprinting; *in situ* polymerization; affinity membranes; monocrotophos; solid-phase extraction

INTRODUCTION

During the last decades, molecular imprinting has received considerable attention as an approach for introducing binding sites mimicking those of biological receptors in synthetic polymers.[1–3] Compared with biosystem such as enzymes and antibodies, molecularly imprinted polymers (MIPs) possess several advantages such as temperature stability, compatibility with organic solvents, reusability, and low cost of preparation. MIPs have been widely studied for chromatographic separation,[4] as antibody mimics,[5] and as selective elements of chemical sensors.[6] In particular, the application of MIPs for solid-phase extraction (SPE) is a field of intense development.[7,8] A typical way of generating MIPs is the bulk polymerization of monomers in the presence of templates, followed by grinding, sieving, and sedimenting the polymers. This procedure requires ~1 week and liters of organic solvents for producing a single polymer.

Recently, MIP membranes, instead of columns filled with particles, have become increasingly attractive for efficient affinity separations.[5,9] The preparation of such membranes has been attempted via immersion precipitation phase inversion; however, those membrane's fluxes are rather low.[10] Moreover, it is difficult to prepare thin and stable membranes with reproducible properties from highly crosslinked polymers. In contrast, membrane modification, by *in situ* polymerization on the surface of the already flux-optimized microfiltration membranes in presence of a template, has been shown to be a feasible novel approach.[11]

Monocrotophos (MCP) is a pesticide that generally acts as cholinesterase inhibitor and is used for the control of a broad range of pests on cotton, rice, tobacco, sorghum, sugarcane, and vegetables. However, MCP is toxic to all animals and humans. For evaluation of environmental samples, highly sensitive methods for the determination of MCP in soil and water are required. Many articles have described the determination of MCP in aqueous samples. Because MCP is highly polar and extremely water soluble, and not extractable (after the adequate pH adjustment), the conventional LLE or SPE procedures are not suitable for MCP.[12,13] So, increasing the selectivity of sorbent in the extraction of analytes and developing new efficient cleanup techniques are highly attractive for monitoring trace MCP in aqueous samples.

The aim of this study was to develop a method for achieving MIP synthesis in a thin layer on the entire surface of a porous microfiltration membrane without

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impairing its porosity and permeability. In this study, novel thin layer composite MIP membranes selective to MCP pesticide were obtained by *in situ* polymerization of methacrylic acid (MAA) as a functional monomer with ethylene glycoldimethacrylate (EGDMA) as crosslinker. A Nylon-6 membrane was found to be especially beneficial for efficient MIP membranes synthesis and good separation (SPE) performance. The novel thin layer MIP composite membranes should introduce specific binding sites into the porous membrane without damaging its pore structure and thus preserving its transport properties. Furthermore, the application potential of the novel MIP composite membranes for a fast SPE of MCP pesticide from aqueous solution was demonstrated.

EXPERIMENTAL

Materials

Nylon microfiltration membranes (NL6) with pore diameter 0.45 μm and a membrane thickness 125 μm were purchased from Schleicher and Schuell (Dassel, Germany). Monocrotophos (MCP; 99.5%), mevinphos (MVP; 99.8%), phosphamidon (PPD; 99.4%), and omethoate (OTT; 99.8%) were purchased from Bai-Ling-Wei Chem-Tech (Beijing, China). Acrylic acid (AA), methacrylic acid (MAA), acrylamide (AAM), and ethylene glycoldimethacrylate (EGDMA) were from Aldrich and were cleaned to remove the inhibitor prior to polymerization. Azobisisobutyronitrile was from Factory of Special Reagent of Nankai University (Tianjin, China). All other chemicals were of analytical grade, and solvents were of HPLC quality. Ultrapure water used for sample preparation was obtained from a MILLI-R04 purification system, (Millipore, Germany).

Instrumental

Chromatographic evaluation was performed on an Agilent 1100 series high performance liquid chromatography equipped with 1312A Binary Gradient Pump, 1313A Thermostatted Autosampler, G1316A column oven, G1315A Diode Array Detector, and G1319A Chemstation. Chromatographic separation was carried out with an Agilent XDB-C_{18} column (250 mm $\times$ 4.6 mm *i.d.*; particle size 5 μm). The eluent was acetonitrile/water (20:80, v/v), and detection was carried out at 240 nm. The column was thermostated at 25°C.

A JEOL model JSM-6700F scanning electron microscope (SEM) was used to visualize the surface features of the film. A thin layer of gold was coated before the SEM analysis.

TABLE I
The Material and Amount Used for Casting MIPs on Nylon Membranes within Microfiltration Modules

Function	Component	Amount
Template	MCP	1×10^{-5} mol
Functional monomer	MAA, AA, or AAM	4×10^{-5} mol
Crosslinker	EGDMA	20×10^{-5} mol
Porogen	CH_2Cl_2, ACN, or Toluene	20 μL
Initiator	AIBN	2.0×10^{-6} mol

Synthesis of thin layer MIP composite membranes

Circular nylon membrane samples (4.9 cm^2 area) were cleaned by pumping 3 L of methanol for all the modules in series using an automatic pump and weighed before use.

In all the recipes, 10^{-5} mol of template (MCP) was used. Prepolymerization solutions (as shown in Table I) were mixed for 30 min at room temperature before rinsing through labeled weighed, and cleaned Nylon membranes within single-use modules using 1 mL syringes. The modules were then flushed gently with nitrogen for 15 min to remove the excess mixture and oxygen before being closed from both sides with a sealed canula at the outlet and a 1-mL syringe at the inlet. Polymerization was performed by heating the modules in an oven at 65°C overnight. After polymerization, the modules were washed with a 10:1 methanol–acetic acid solution to extract the template, rinsed with methanol to eliminate residual acetic acid using an automatic pump, dried at 50°C, and weighed again. The degree of modification (DM) was calculated from the weight differences of the modified and unmodified membranes. The blank polymer membranes were generated and processed in the same way, but in the absence of any template.

Affinity evaluation of MIP composite membranes

The membrane recognition properties were evaluated by measuring their capacity to adsorb MCP from aqueous solution during a fast filtration (membrane-SPE). Sorption was measured using a syringe connected to the membrane filter coated with MIP and blank films. Ten milliliter of 10^{-5} mol/L MCP solution in water was filtered through the membranes at a rate of 5 mL/min. Then the dried membranes were submitted to a washing step, which was carried out with 10 mL of chloroform, water, dichloromethane, or methanol. Next, the membranes were extracted with 10 mL of methanol. The MCP concentration in feed, permeate, washing, and elution fraction were analyzed by reversed-phase HPLC system.

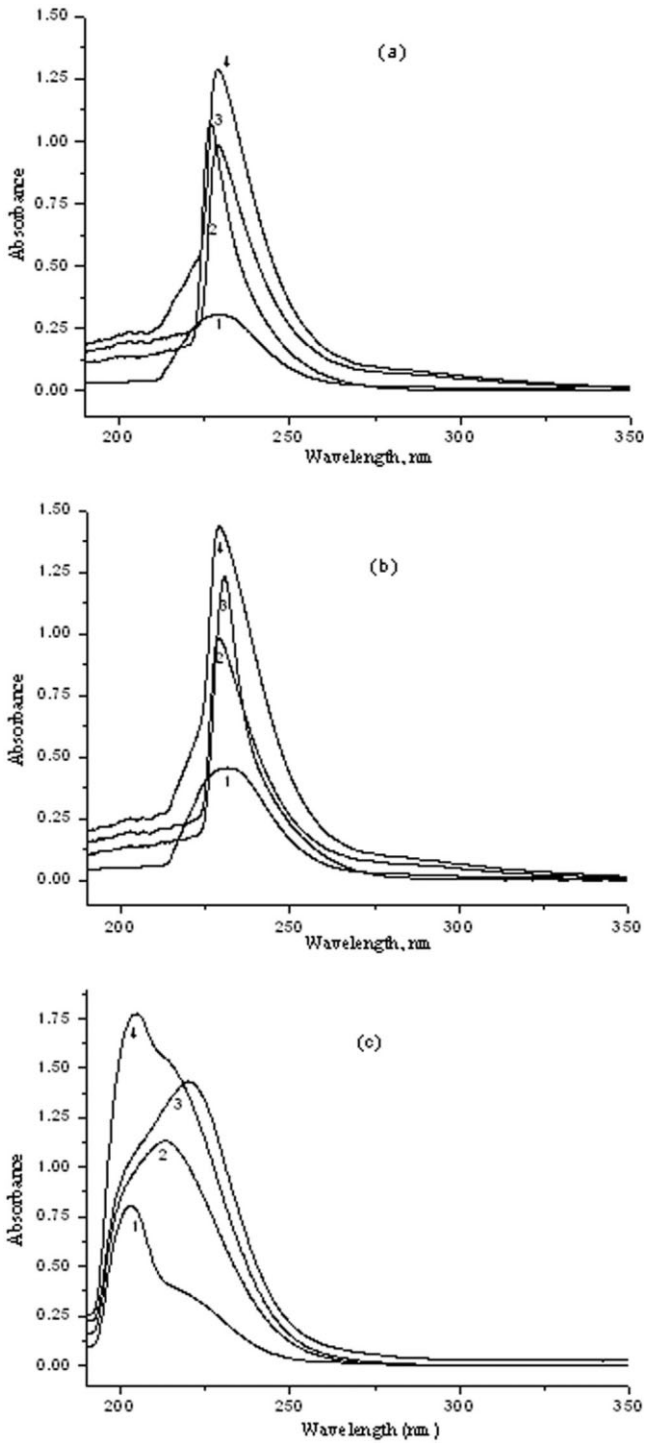

Figure 1 UV spectra for MCP (10^{-4} mol/L) and functional monomers ((a) AA and (b) MAA in dichloromethane; (c) AAM in acetonitrile) related to noncovalent complex formation in the reaction mixture used for MIP synthesis. 1, monomer (4×10^{-4} mol/L); 2, MCP (10^{-4} mol/L); 3, mixture; 4, theoretical sum.

RESULTS AND DISCUSSION

The imprinting effect is generally believed to result from the complexation between template and functional monomers. Fixation of the structure of these complexes in a rigid polymer network formed during the polymerization process produces recognition sites containing polymeric functionalities positioned to complement those of the template molecule. Here, the development of a surface functionalization of a porous membrane with a MCP-imprinted MIP layer from organic imprinting mixtures is the main objective.

Selection of the functional monomer

When template and functional monomers form complexes in solution, the strength of these complexes is reflected in the affinity and selectivity of the imprinted polymer. Functional monomers, giving high degrees of template complexation in a monomer mixture, are supposed to generate polymers that demonstrate high selectivity together with low levels of nonspecific binding. Consequently, the choice of functional monomers is of significant importance for the quality of recognition sites in MIPs. There is amino group in the structure of MCP, which can interact with acid monomer, such as MAA.[14] To justify a comparison between monomers (here AA, MAA, and AAM) and their suitability for creation of recognition sites via molecular imprinting, their ability to noncovalently interact with MCP in a monomer mixture were studied by UV difference spectroscopy.

The titration of 0.1 m*M* solution of MCP in dichloromethane by increasing amounts of AA, MAA, or AAM resulted in significant changes in the UV spectra, which were the superimposition of the absorbances for the single components [as shown in Figs. 1(a)–1(c)]. For every monomer (acetonitrile was chosen as the solvent to optimize solubility of AAM), the observed shift reached a maximum, corresponding to a saturation of interaction between template and functional monomer. The calculated dissociation constant $K_{diss,a}$[15,16] values indicated that the complex MAA-MCP(6.63×10^{-7}) was stronger than AA-MCP (7.45×10^{-5}) and AAM-MCP (3.04×10^{-4}). From Table II, remarkable differences were observed for binding capacity of MIP membranes prepared from different functional monomers. The MIP composite membrane

TABLE II
Influence of Functional Monomer on MCP Sorption for the Membranes

	Acrylic acid (AA)		Methacrylic acid (MAA)		Acrylamide (AAM)	
Monomers	Blank	MIP	Blank	MIP	Blank	MIP
DM[a] (mg/cm^2)	3.22	2.94	3.68	3.55	3.36	3.23
Binding capacity ($\mu g/cm^2$)	0.69	2.09	0.84	4.24	0.81	0.85

[a] Degree of modification.

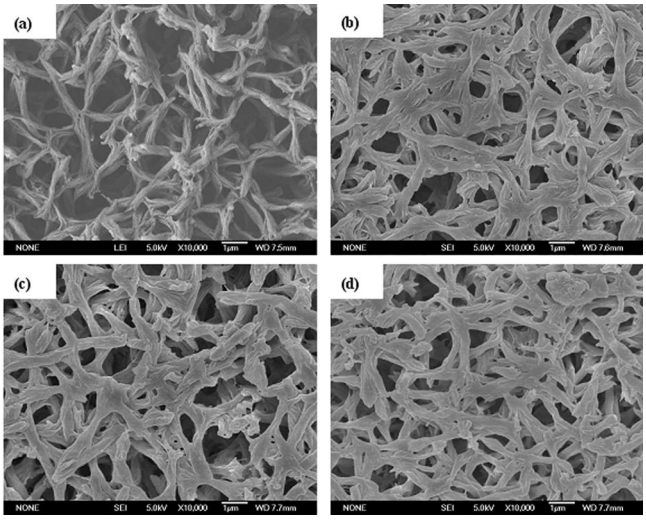

Figure 2 Scanning electron micrographs of the MIP coatings synthesized using different solvents (porogens) (a) Nylon-6 membrane, (b) DCM-MIP, (c) ACN-MIP, and (d) TOL-MIP.

made from MAA was shown highest affinity for MCP when compared with the other two MIP membranes. These results were in agreement with the $K_{diss,a}$ values. Of three functional monomers, it can assumed that MAA ($pK_a = 4.65$) can interact with amino group of MCP and that a hydrogen-bonding complex was formed between template and MAA in dichloromethane.[17] For another acidic monomers AA ($pK_a = 4.2$)[18] and neutral monomers AAM, this mechanism is less effective.

Selection of the porogen

The principle of molecular imprinting lays in the preservation of the prepolymerized host–guest structure into a polymer matrix, and it is crucial that the template and the functional monomers form stable host–guest complexes in the prepolymerization mixture. It has been previously shown that MCP can hydrogen bond with MAA.[14] However, these bonds can be influenced by the porogen. On the other hand, morphology (pore structure) of the MIP coating is very important to the permeabilities of membrane. Therefore, the effect of using different solvents (porogens) for the MIP coating synthesis was studied. Dichloromethane, acetonitrile, and toluene were used as solvents in identical prepolymerization mixtures. In all the cases, a MIP coating with smooth appearance was formed. With SEM, it was shown that Nylon-6 membrane consisted of flexible hydrogen-bonding networks with regular cavity [Fig. 2(a)], and that the similar rigid crosslink structure polymer with visible pores could be detected in three MIP thin coatings [Fig. 2(b–d)]. The MIP coating synthesized using acetonitrile (ACN-MIP) as porogens appeared larger pores than in the case of dichloromethane and toluene. On the other hand, the MIP coating obtained employing dichloromethane (DCM-MIP) as the porogen appeared to consist of denser cluster units and less pore structures than in the case of acetonitrile and toluene. This is in agreement with Refs. 19 and 20. In addition, no changes of pore morphology can be identified from the MIP and its respective blank membranes (not shown) by SEM analyses.

Apart from the morphology differences of MIP coatings, the binding capacity of these coatings was also studied. The results (Table III) indicate that the TOL-MIP and DCM-MIP composite membranes had higher affinity for the template when compared with the respective blank membranes, while the ACN-MIP composite membrane as well as its blank membrane showed negligible binding of MCP. These findings could be interpreted as follows. It was well known that the molecular recognition principle of most of MIPs was based on the hydrogen binding between the target and the polymer functional groups, which often occurred in aprotic and low polar organic solvents.[21] Thus, the solvent can be chosen not only to optimize solubility of monomers and template molecule but also to govern optimal monomer–template interaction in the prepolymerization mixture, resulting in well-defined molecular imprints of high yield. Toluene and dichloromethane were solvents with poor hydrogen bonding capacity that can promote

TABLE III
Influence of Porogens on MCP Sorption for the Membranes

Membranes	Porogens	DM[a] (mg/cm^2)	Binding capacity ($\mu g/cm^2$)	Water permeabilities ($L/(m^2\ h\ bar)$)
Blank1	Acetonitrile	3.09	0.93	11,200
MIP1	Acetonitrile	2.30	0.77	11,500
Blank2	Dichloromethane	2.74	0.84	8,800
MIP2	Dichloromethane	2.57	4.24	9,100
Blank3	Toluene	4.02	0.89	9,900
MIP3	Toluene	3.51	4.26	10,300
Nylon-6[b]	No	0	0.04	12,100

[a] Degree of modification.
[b] The comparison for the water permeability.

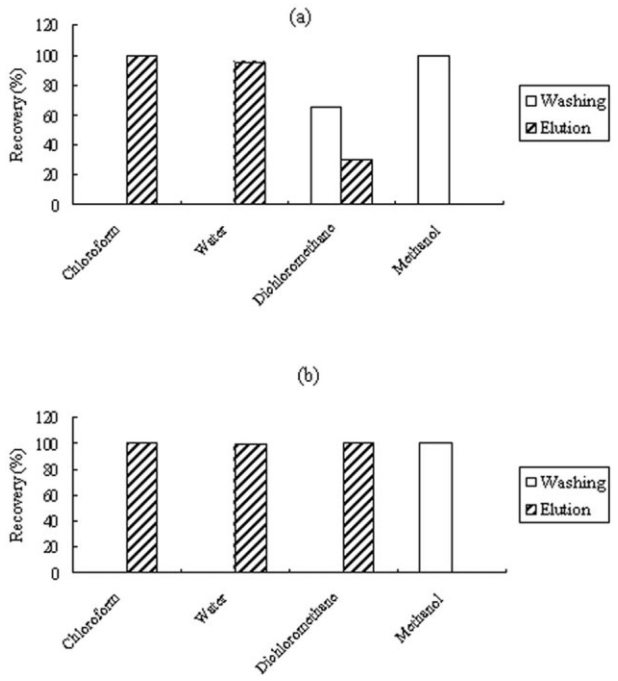

Figure 3 Recovery of MCP in the washing (open bars) and elution (shades bars) fractions after loading 10 mL of 10^{-5} mol/L MCP solution on blank membrane (a) and MIP membrane (b). Washing step: 10 mL of each of the solvents in the figure. Elution step: 10 mL of MeOH.

strong binding between monomer and template.[19] To sum up the pore structure and binding capacity of membranes, toluene seemed to be most the efficient solvent for the MIP coating synthesis.

On the other hand, with SEM, no changes of the membrane pore structure due to functionalization could be detected. Hence, the coverage of entire membrane surface with a very thin MIP coating can be assumed without membrane pore blocking by excess polymer. The same results (as shown in Table III) can be obtained from the test of water permeabilities.[22] Of course, some reductions of water permeabilities could be observed with very high DM value.

Determination of MCP elution conditions for MIP membrane SPE

For optimizing the conditions of the washing step, solvents were studied using the MIP in a membrane solid phase extraction. First, a standard solution of MCP (10^{-5} mol/L in water) was applied to the MIP and blank membrane. Second, the MIP and the blank membranes were submitted to a washing step, which was carried out with 10 mL of either chloroform, water, dichloromethane, or methanol. Last, the membranes were eluted with 10 mL of methanol. Both the loading, washing, and elution fractions of the solvent were collected and analyzed by reversed-phase HPLC. The results were shown in Figure 3. It can be seen that most of the MCP was still retained on the blank membrane after it was washed using 10 mL of chloroform. Therefore, the low polar organic solvent (chloroform) cannot disrupt the nonspecific binding between the polymer and MCP. On the contrary, the MCP nonspecifically adsorbed on the blank polymer can be efficiently removed using high polar solvents (methanol). However, the specific interaction between the polymer and MIP was also suppressed by the use of these polar solvents in the washing step. It has shown that MCP can hydrogen bond with MAA. And these bonds can be disrupted by polar solvents. Therefore, it is possible that in methanol and water, MCP hydrogen bonds with the solvent and decreases its interaction with the MIP. The high nonspecific binding that we observed with water was most likely the hydrophobic effect of polymer.[23] On the other hand, while using dichloromethane as washing solvent, a different result was observed. About most of the amount of MCP loaded on the blank membrane was washed off using 10 mL of this solvent. However, the MCP was still selectively retained on the MIP membrane after the washing step and then quantitatively eluted by methanol. For the elution solvent, hydrogen bonding was significantly weakened because of the interference of methanol.

Affinity and specificity of the membrane

The optimized conditions of a fast SPE (membrane-SPE) were applied to characterize membrane/solute affinity. MCP binding to the functionalized membranes was estimated in filtration experiments using a solute concentration of 10^{-5}mol/L in water. In all the cases, MIP membranes demonstrate higher MCP sorption in comparison with blank ones. As shown in Table IV, it can be seen that most of the MCP was washed off after it had been washed using dichloromethane in blank membrane while MCP was still selectively retained on the MIP membrane and then extracted by methanol. It could be concluded that orientation of the polymer functional groups in the imprinted receptor site was suited for high specific binding of MCP that permitted to reach an effective

TABLE IV
Recoveries of MCP (10^{-5} mol/L) by Membrane-SPE using Aqueous Solution Loading, Washing, and Elution Steps (n = 3)

		Recovery (%)	
	Fractions	MIP (% ± SD)	Blank (% ± SD)
Loading	10 mL aqueous solution	n.d.[a]	n.d.
Washing	10 mL dichloromethane	n.d.	51.2±5.6
Elution	10 mL methanol	91.8±3.2	34.5±4.3

[a] Not detected.

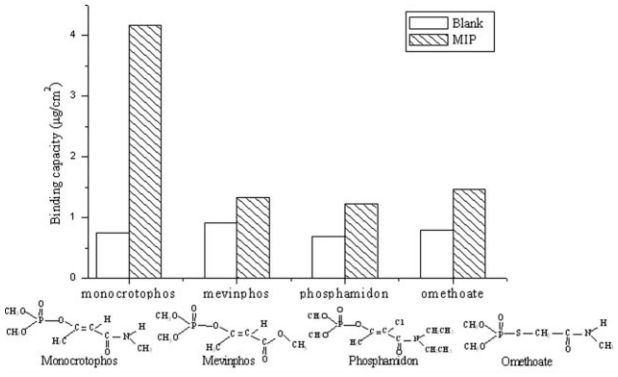

Figure 4 Selectivity of the MCP-imprinted thin-layer MIP composite membrane to other organophosphorus pesticides of related chemical structure. The membranes were synthesized with MAA as functional monomer and toluene as porogen. Solutions (10^{-5} mol/L) of pesticide in water were used in (SPE) filtration experiments.

removal of MCP from aqueous solution. Blank membrane at the same conditions had a less affinity to MCP in comparison with MIP sample. Hence, sorption onto the blank membrane from aqueous solution was driven by hydrophobic interaction, which can easily be suppressed by dichloromethane.[20] In addition, it could be seen that the recovery (85.7%) of the blank membrane was lower than that of on the MIP (91.8%). When loading the MCP solution in water on the blank and MIP membranes, almost all of MCP was retained on the blank and MIP membranes except little leak of MCP from the blank membrane. This was in agreement with the Ref. 24. Optimal MIP membranes (TOL-MIP) showed a high sorption of MCP (>90%) at high water flux (400 L/m^2 h per bar).

The membrane selectivity was evaluated in filtration experiments using four different organophosphorus pesticides: MCP, MVP, PPD, and OTT (Fig. 4). In all the tests, a solute concentration of 10^{-5}mol/L in water was applied. Remarkably, the novel MIP composite membrane as well as the blank membrane had lower binding of the other pesticides in comparison with the very efficient sorption of the template MCP. This could be easily explained by their structural homology to MCP. From Figure 4 it can be seen that there are some differences between the structure of MCP and those of MVP, PPD, and OTT. For MVP, the structural difference is a —O instead of —N—H. For PPD, the structural difference is a —Cl instead of —H in the —C═C position and two ethyl instead of —H and methyl in the —N position. The size of —Cl and ethyl is bigger than that of H and methyl, respectively. This further demonstrates that the imprinting is not only based on the interaction of the functional groups of the analyte with those binding sites in the polymer cavities but also based on the combined effect of shape and size complementarily.[25] Therefore, the novel MIP composite membranes had not only higher affinity for the template as compared with the respective blank membranes but they also recognized this template with a high selectivity compared to other similar substances.

CONCLUSIONS

A new type of thin-layer MIP composite membranes for selective binding of MCP pesticide were prepared as deposits on the surface of Nylon-6 membranes by *in situ* polymerization. By systematic optimization of synthesis conditions such as the type of functional monomer and porogen, a MIP membrane with high affinity and low nonspecific binding as compared with control blank membrane could be obtained. With the optimized membrane SPE conditions, the novel MCP-imprinted membranes demonstrated much higher sorption capability to this pesticide than to structurally similar compounds.

The high affinity of the novel MIP composite membranes to MCP together with their simple and inexpensive preparation provides a good basis for practical applications such as a preconcentration step for the determination of polar organophosphorus pesticides in environmental analysis.

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