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Journal of Chromatography A, 1092 (2005) 161–169
JOURNAL OF CHROMATOGRAPHY A
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Selective solid-phase extraction using molecularly imprinted polymer for the analysis of polar organophosphorus pesticides in water and soil samples

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Received 16 May 2005; received in revised form 7 July 2005; accepted 8 July 2005
Available online 15 August 2005

Abstract

An analytical methodology for the analysis of four polar organophophorus pesticides (monocrotophos, mevinphos, phosphamidon, omethoate) in water and soil samples incorporating a molecularly imprinted solid-phase extraction (MISPE) process using a monocrotophos-imprinted polymer was developed. Binding study demonstrated that the polymer showed excellent affinity and high selectivity to monocrotophos. The MISPE procedure including the clean-up step to remove any interferences was optimized. The accuracy and selectivity of the MISPE process developed were verified using a non-imprinted (blank) polymer and a classical ENVI-18 cartridge as the SPE matrix during control experiments. The use of MISPE improved the accuracy and precision of the GC method and lowered the limit of detection. The recoveries of four polar organophosphorus pesticides (OPPs) extracted from 1 L of river water at a 100 ng/L spike level were in the range of 77.5–99.1%. The recoveries of organophosphorus pesticides extracted from a 5-g soil sample at the 100 μg/kg level were in the range of 79.3–93.5%. The limit of detection varied from 10 to 32 ng/L in water and from 12 to 34 μg/kg in soil samples. The molecularly imprinted polymer (MIP) enabled the selective extraction of four organophosphorus pesticides successfully from water and soil samples, demonstrating the potential of molecularly imprinted solid-phase extraction for rapid, selective, and cost-effective sample pretreatment.
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Keywords: Solid-phase extraction; Organophophorus pesticides; Monocrotophos; Water and soil samples; Molecularly imprinted polymers

1. Introduction

Organophosphorus pesticides (OPPs) are a class of pesticides that generally act as cholinesterase inhibitors and are used for the control of a broad range of pests on cotton, rice, tobacco, sorghum, sugarcane and vegetables [1]. However, OPPs are toxic to all animals and humans. For evaluation of environmental samples, highly sensitive methods for the determination of OPPs in soil and water are required.

Many papers have described the determination of OPPs in aqueous samples. Most OPPs are easily analyzed by GC and HPLC [2–4]. Generally, the trace analysis of complex samples (e.g., environment and biological samples) needs a pretreatment step in order to reduce the matrix interference and enrich the analytes. This is often performed by solid-phase extraction (SPE) [5,6]. This technique is more rapid, simple, and economical than the traditional liquid–liquid extraction (LLE). By use of SPE, the detectability of diluted analytes can be greatly enhanced by applying large sample volumes. SPE using C_{18}-silica sorbents and other similar matrixes is today often used in environmental analysis for sample enrichment of OPPs [7,8]. These sorbents retain the analytes primarily by hydrophobic interactions and are thus fairly nonspecific in nature.

Organophosphorus pesticides vary widely in physicochemical properties like water solubility, K_{ow}, vapour pressure, absorb constant in soil, molecular mass and thermal

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doi:10.1016/j.chroma.2005.07.037

Table 1
Basic physico-chemical properties of organophosphorus pesticides [2,9]

Pesticides	M_w	Solubility (in H_2O) (g/L)	Soil half life (days)	Vapour pressure (mPa at 25 °C)	Pesticide movement	Absorb constant in soil[a] (L/kg)
Monocrotophos	223.2	1000	30	0.98	Very high	1
Mevinphos	224.2	600	3	1.733	High	44
Phosphamidon	299.7	1000	17	0.213	High	7
Omethoate	213.2	1000	15	6.33	High	5

[a] Absorb constant in soil: the ratio of amount of pesticides absorbed in soil and concentration in water in an equilibrium system of water and soil.

stability. A small number of OPPs with similar properties, however, are missing in the methods described in literature or only incidentally taken into consideration, and include: monocrotophos (MCP), mevinphos, phosphamidon and omethoate (selected physico-chemical properties and structures are presented in Table 1 and Fig. 1). These OPPs have in common that they are highly polar and extremely water soluble, not extractable (after the adequate pH adjustment) using the conventional LLE or SPE procedures [5,9]. So, increasing the selectivity of sorbent in the extraction of analytes and developing new efficient cleanup techniques are highly attractive for monitoring trace analytes in complex samples.

A novel, high selectivity approach is presented, by using molecularly imprinted polymers (MIPs) for the cleanup and preconcentration of compounds from complex matrix. During molecular imprinting, cross-linked polymers are formed by free-radical copolymerization of functional monomers with an excess of cross-linker around an analyte that acts as a template. After removal of the template molecule, the polymer can be used as a selective binding medium for the template molecule or structurally related compounds. The mechanisms by which these polymers specifically bind the print molecule and related ligands are attributed to the formation of functional groups in a specific arrangement within the polymer that corresponds to the template molecule and to the presence of shape-selective cavities. MIPs have been exploited in a number of applications including their use as separation materials [10], as antibody mimics [11] in binding assay systems, and as recognition elements in biosensors [12] for assay of various analytes. Recently, because of their compatibility with organic solvents, MIPs have attracted considerable attention as SPE sorbents for the cleanup and preconcentration of target analytes prior to determination. To date, molecularly imprinted solid-phase extraction (MISPE) has been applied to determine bentazone [13], simazine [14], nitrophenol [15], pirimicarb [16], atrazines [17] and sulfonylureas [18] in environmental samples. However, the use of MIPs as separation materials for enriching OPPs from environmental samples has not been reported so far.

In this paper, a MIP was synthesized using MCP as the template molecule, methacrylic acid as a functional monomer and ethylene glycol dimethacrylate as a cross-linker. After polymerization, MISPE was used for the selective preconcentration of four polar OPPs from environmental samples prior to chromatography analysis and compared to results obtained with commonly used reversed octadecyl silane (ENVI-18) stationary phases and LLE. The major advantages of this method are that MIP shows high selectivity and affinity to the target analytes and is very stable for a real environmental application. To our knowledge, MIPs against any of the OP compounds have not been prepared before. The present study is the first work described a method for the determination of trace polar OPPs in real environmental samples with MISPE enrichment.

2. Experimental

2.1. Chemicals

Monocrotophos (99.5%), mevinphos (99.8%), phosphamidon (99.4%), and omethoate (99.8%) were purchased

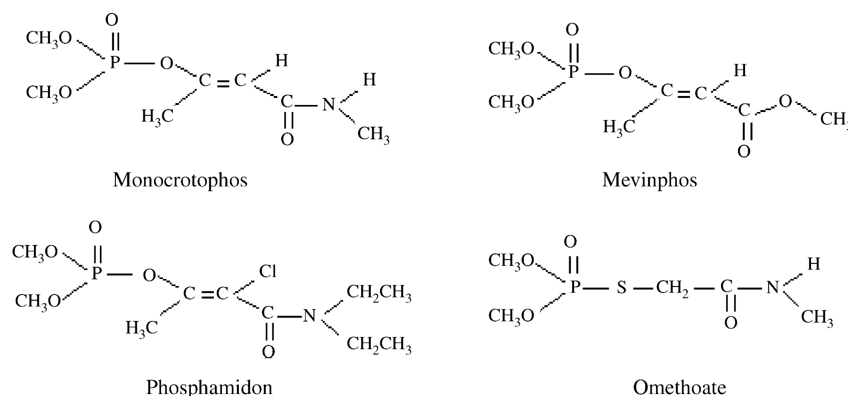

Fig. 1. The chemical structures of monocrotophos, mevinphos, phosphamidon and omethoate.

from Beijing Bai-Ling-Wei Chem-Tech. Methacrylic acid (MAA) and ethylene glycoldimethacrylate (EGDMA) were from Aldrich and cleaned to remove the inhibitor prior to polymerization. Azobisisobutyronitrile (AIBN) was from Factory of Special Reagent of Nankai University. 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).

2.2. Preparation of MIP

For polymer preparation, 223.2 mg (1 mmol) of template (MCP) and 4 mmol of MAA were dissolved in 5.6 mL of dichloromethane in a 20 mL glass tube. The EDGMA cross-linker (20 mmol) and the AIBN initiator (40 mg) were added to the mixture and purged by nitrogen for 10 min. The tube was sealed under vacuum and placed in a shaker bath at 58 °C for 24 h. As a reference, a nonimprinted polymer was simultaneously prepared in the same way but without the addition of the template.

The bulk polymer obtained was crushed, ground and wet-sieved with acetone. The particle size fraction of 40–60 μm was collected. The resulting particles were placed in a Soxhlet extraction apparatus and washed with 10% acetic acid methanol solution until MCP could no longer be detected at 220 nm in the eluent. Then the particles were washed with methanol to remove residual acetic acid and dried to constant weight under vacuum at 70 °C.

2.3. ^{1}H NMR study

The ^{1}H NMR spectra study was carried out with FT-NMR Model AV 400 (Bruker, Switzerland). Samples were prepared with a fixed concentration of MCP (20 mmol/L) and varying concentration of MAA (from 0 to 200 mmol/L) in CD_2Cl_2. TMS was used as an internal standard. The measurements were carried out at 20 °C.

2.4. Instrument and chromatographic conditions

HPLC: chromatographic evaluation was performed on an Agilent 1100 series high performance liquid chromatography equipped with a 1312A binary gradient pump, a 1313A thermostatted autosampler, a G1316A column oven, a G1315A diode array detector and a G1319A Chemstation. Chromatographic separation was carried out with an Agilent XDB-C_{18} column (250 mm × 4.6 mm I.D., particle size 5 μm).

The GC system consisted of a TRACE gas chromatograph from Finnigan (USA) equipped with an AI3000 Autosampler, a split/splitless injector and a nitrogen-capture detection (NPD) system. The capillary column was DB-5, 30 m × 0.25 mm I.D. and coated with a film thickness of 0.25 μm (Agilent, USA). Helium was used as the carrier gas at flow rate 2.0 mL/min. The column temperature was programmed at 120 °C for 3 min, raised to 250 °C at 20 °C/min and the final temperature was held for 4 min. The detector gases were: air 60 mL/min, hydrogen 2.3 mL/min and nitrogen (make-up) 15 mL/min. All the samples were operated in the splitless mode (2 μL injection, split after 1.0 min). Detector temperature and injector port temperature were 280 °C and 220 °C, respectively. Chromatographic data acquisition and processing were carried out with Chrom-Card software.

2.5. Binding study of MIPs and Scatchard analysis

The sized and washed polymer particles (10.0 mg) were mixed with a 1.0-mL acetonitrile solution of MCP of varied concentrations from 12.5 μmol/L to 2.5 mmol/L. The mixture was incubated with continuous stirring at 25 °C for 24 h. Following centrifugation, the supernatants were analyzed by reversed-phase HPLC to quantify the concentration of free MCP [MCP]. The eluent was acetonitrile/water (20:80, v/v), and detection was carried out at 220 nm. The amount of MCP bound to the polymer, Q, was calculated by subtracting [MCP] from the initial MCP concentration. The average data of triplicated independent results were used for the Scatchard analysis.

Binding data can be linearly transformed according to the Scatchard equation [19], $Q/[\mathrm{MCP}] = (Q_{\max} - Q)/K_D$, where K_D is an equilibrium dissociation constant and $Q_{\max}$ is an apparent maximum number of binding sites. When $Q/[\mathrm{MCP}]$ is plotted versus Q, K_D and $Q_{\max}$ can be estimated from the slope and the intercept, respectively.

2.6. MISPE cartridges preparation, washing, and elution procedures

A slurry of 200 mg of MIP in 1.0 mL of MeOH was placed into an empty PTFE SPE cartridge (3-mL cartridge from Supelco, Shanghai, China). PTFE frits (porosity 20 μm, Supelco) were placed above and below the sorbent bet. Prior to and between uses, the columns were washed successively with 10% (v/v) acetic acid/acetonitrile (10 mL), acetonitrile (20 mL), and dichloromethane (20 mL). Before the samples were processed, the cartridge was preconditioned with 1 mL of MeOH and 2 mL of LC-grade water. As a control, a blank SPE column was also prepared in the same manner but with the blank polymer.

A 1.0-mL sample of 1 μg/mL MCP standard solution was passed through at a flow rate of ∼1 mL/min, then the cartridge was washed with 2 mL of a CH_2Cl_2/ACN solution (95:5, v/v). The analytes retained in the cartridge were eluted with 2 mL of CH_2Cl_2/MeOH (90:10, v/v). Both the washing and elution fractions were collected and dried using a gentle stream of nitrogen. The residues were redissolved in 1.0 mL dichloromethane and analyzed by GC/NPD.

2.7. Water sample preparation and MISPE extraction

Surface water was collected from river in south China and filtered using glass fiber filter (from Dikma, Beijing, China) to remove particles large than 0.45 μm and kept at 4 °C until analysis. The drinking water sample was collected from the

tap in the laboratory. For recovery studies, surface water and drinking water samples were spiked with 0.5 mL of standard solution (1.0 μg/mL of each of four OPPs).

Prior to sample application, the cartridge was conditioned with 1 mL of MeOH and 2 mL of LC-grade water. A total of 1 Lof each sample was forced to pass through the MISPE cartridge at a flow rate of ∼10 mL/min by negative pressure. After the sample was passed through the cartridge, the cartridge was dried with a nitrogen stream for 20 min. Then the cartridge was washed and eluted under optimal solvents. Both the washing and elution fractions were collected and dried using a gentle stream of nitrogen, and the residue was reconstituted with 1.0 mL dichloromethane and analyzed by GC/NPD. As a control, the sample extraction was simultaneously applied on a blank polymer SPE cartridge in the same manner.

2.8. Soil sample preparation and MISPE

The soil used was collected from dry land of Yongan county (Fujian, China). The sample was ground to a fine powder before use. Fortified samples were prepared by adding 0.5 mL of standard solution (1.0 μg/mL of each of four OPPs) to 5.0 g soil. Additional acetone was added until the solvent completely covered the soil particles. The spiked sample was allowed to stand overnight before extraction. Fortification was made at 100 μg/kg.

The extraction of soil sample was performed in the following way [6]: a 5.0 g spiked moist soil sample was added to 5 mL 50 °C distilled water and 5 mL acetone. Then the mixture was shaken by sonication for 15 min and the slurry was centrifuged at 4000 rpm for 10 min. The extraction procedure was repeated three times, and the liquid extracts were incorporated. Then, the supernatant was decanted into a reservoir containing approximately 100 mL distilled water and passed through the MISPE cartridge. The washing, elution, and analytical procedures were the same as described above. An unspiked (blank) soil sample was also extracted and analyzed. Furthermore, a blank polymer SPE cartridge was simultaneously applied in the same manner.

A dichloromethane liquid–liquid extraction (LLE) was performed in the following way [8]: a mixture of soil extract and 100 mL water was extracted with 3 × 50 mL of dichloromethane. The combined organic extracts were filtered throughout a thin layer of anhydrous sodium sulfate and concentrated by a rotary evaporator until 2–3 mL. This extract so obtained was again evaporated to dryness with gentle stream of nitrogen and redissolved in 1.0 mL dichloromethane before injection.

3. Results and discussion

3.1. Recognition mechanism

The study of recognition mechanism of the polymer would be important to understand the imprinting and recognition phenomena. Therefore, the reaction mixture was investigated by ^{1}H NMR. Since the cross-linker and the initiator would be much less important for the interaction of the template and the functional monomer, the NMR study was performed with diverse molar ratios of the template MCP and MAA in CD_2Cl_2. In this system, the amino group and phosphate ester group of MCP could presumably interact with the carboxyl group of MAA. As expected, the addition of MAA into the MCP solution resulted in low-field shift of the peaks of proton of amino group of MCP derived from 6.001 (20 mmol/L MCP pure solution) to 7.421 ppm (20 mmol/L MCP in the presence of 200 mmol/L MAA) (as shown in Fig. 2). The observation suggests that proton of amino group of MCP is involved in hydrogen-bonding formation [19]. Because it would be important to provide multipoint interacting binding sites of high selectivity in the resulting polymer, excess (4 equimolar) of MAA was added to the template for the polymer preparation in this study. Dichloromethane was carefully chosen as the solvent because it does not interfere with hydrogen bonding.

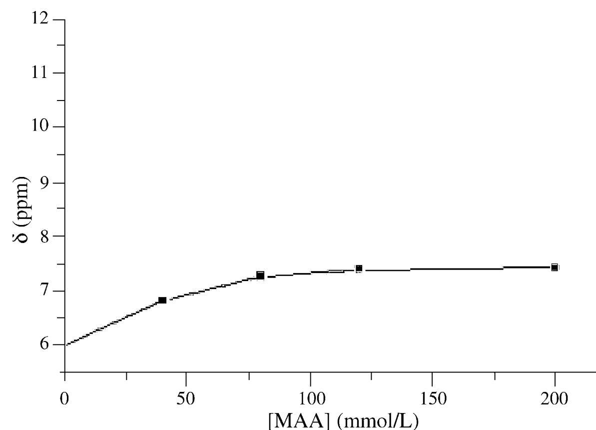

Fig. 2. The effect of the addition of MAA on the chemical shift of amino proton of MCP on ^{1}H NMR in CD_2Cl_2 at 20 °C (MCP: 20 mmol/L).

3.2. Affinity of the MCP-imprinted polymer

In order to investigate the binding performance of the MCP-imprinted polymer P(MCP), saturation experiments and subsequent Scatchard analysis were carried out. As shown in Fig. 3, the Scatchard plot was not linear, suggesting that the binding sites in P(MCP) are heterogeneous in respect to the affinity for MCP [20]. Because there are two distinct sections within the plot which can be regarded as straight lines, it would be reasonable to assume that the binding sites can be classified into two distinct groups with specific binding properties. Under this assumption the respective K_D values can be calculated to be 30.0 μmol/L and 557 μmol/L, and the respective Q_{max} 4.0 μmol/g and 9.20 μmol/g of dry polymer. The obtained values for Q_{max} would therefore correspond to 17.5% and 39.8% of the theoretical total binding sites [21]

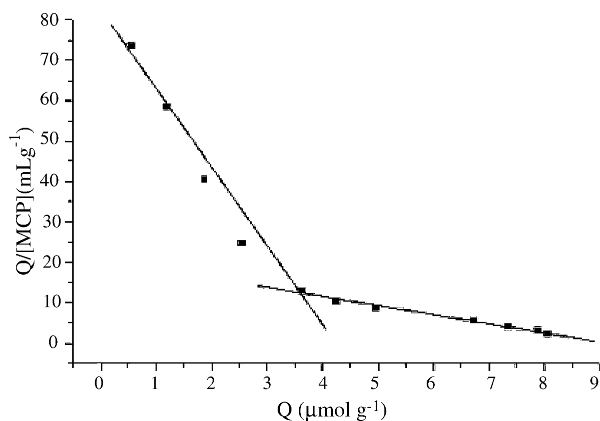

Fig. 3. Scatchard plots to estimate the binding nature of P(MCP).

derived from the amount of the template used for the polymerization.

3.3. Determination of MCP elution conditions for MISPE columns

The binding properties of molecularly imprinted sorbent are influenced by the type of solvent, or porogen, used in polymer synthesis and the solvent used in the particular application of the MIP [22]. Therefore, solvents were studied using the MIP in a solid-phase extraction column. For optimizing the conditions of the washing step, a standard solution of MCP was applied to the MISPE and blank polymer cartridges. First, the MISPE and the blank polymer columns were submitted to a washing step, which was carried out with 2 mL of either chloroform, water, dichloromethane, acetonitrile, or methanol. Next, the cartridges were eluted with 2 mL of methanol. Both the washing and elution fractions of the solvent were collected and analyzed by reversed-phase HPLC. The results were shown in Fig. 4. It can be seen that almost all of the MCP was still retained on the blank column after it was washed using 2 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 and acetonitrile). However, the specific interaction between the analyte 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 we observed with water was most likely the hydrophobic effect of polymer [23]. On the other hand, when using dichloromethane as washing solvent, a different result was observed. About 40% of the amount of MCP loaded on the blank cartridge was washed off using 2 mL of this solvent. However, the MCP was still selectively retained on the MIP cartridge after the washing step and then quantitatively eluted by methanol.

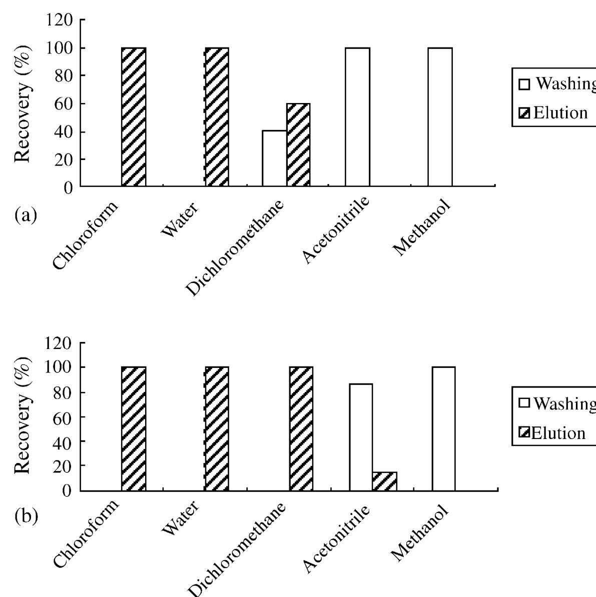

Fig. 4. Recovery of MCP in the washing (open bars) and elution (shades bars) fractions after loading 1.0 mL of 1.0 µg/mL MCP solution on blank polymer (a) and MISPE cartridges (b). Washing step: 2 mL of each of the solvents in the figure; elution step: 2 mL of MeOH.

The mixtures of dichloromethane with different concentration of ACN were tested as washing solvent. Table 2 showed the recoveries of MCP in the washing and elution fractions after preconcentration on the blank and MIP cartridge by using 2 mL of each of the washing solvents. According to the table, when the concentration of ACN in dichloromethane was in the range of 5–6%, the analyte nonspecifically loaded on the blank cartridge was completely removed after the washing step, whereas the specific binding of analyte on the MIP column was still retained. In fact, wash with dichloromethane solution where the system was changed to a hydrogen bonding-based affinity mode in which MCP can be selectively retained in the polymer while other structurally unrelated impurities were washed off [24]. For this reason, 2 mL of 5% of ACN in dichloromethane was selected as the washing solvent for all further experiments.

For the elution solvent, hydrogen bonding is significantly weakened because of the interference of methanol. On account of the swelling properties of solvents for the MIP [25], an elution solvent of dichloromethane containing 10% methanol was chosen in this work as elution solvent to ensure the recovery. Zhu et al. [18] have reported the optimal washing and elution solvents of MISPE for sulfonylurueas. These results showed that the elution conditions of MISPE for MCP were similar to those. It is well known that the molecular recognition principle of most of MIPs is based on the hydrogen binding between the target and the polymer functional groups. There is amino group in the structures of both MCP and metsulfuron-methyl, which can interact with monomer (MAA or TFMAA) by hydrogen bond [26]. Therefore, the strength of these interactions during the recognition process would be affected by the polarity of solvents. These results

Table 2
Recovery of MCP in the washing and elution fractions on blank polymer and MISPE cartridge in dependence on the ACN concentration in the washing solution (MCP: 1.0 μg/mL × 1.0 mL)

ACN (%)			1	2	3	4	5	6	7	8	9	10
Blank	Washing	40.3	48.5	526	60.2	71.9	89.6	94.0	94.1	97.6	97.2	97.9
	Elution	58.7	50.5	45.9	37.6	25.9	9.2	6.3	4.7	1.4	0.3	0.3
MIP	Washing	0	0	0	0	2.8	4.8	6.2	29.2	46.1	65.9	82.9
	Elution	98.2	97.8	98.5	98.2	96.7	94.0	92.8	68.9	51.5	31.1	15.8

Washing step: 2 mL of washing solvent (ACN/CH_2Cl_2 mixture); elution step: 2 mL of MeOH.

were also in agreement with the elution conditions of MISPE for atrazine [10].

3.4. Effect of sample pH

The effect of the sample pH on the extraction process was also investigated using a 0.08 mol/L citric acid–0.04 mol/L Na_2HPO_4 buffer between pH 2.0 and 9.0, and processing in MISPE system 1 mL of a 0.2 μg/mL MCP. The cartridge was treated under optimal solvents and the recovery of MCP was calculated. The results showed that the recovery of MCP was about 90% with the pH value from 2.5 to 8.2. The lower recovery at pH 2.0, indicating an analyte breakthrough during the deposition stage, can be explained by the protonation of the MCP molecules. These protonated charged molecules cannot "fit" the binding sites and cannot be adsorbed by the uncharged polymer. The lower recovery at pH 9.0 can be explained by the unstability of MCP in basic solution [27]. As a result, the subsequent analyses were all performed in neutral solution.

3.5. Specificity of the MIP

To evaluate cross-reactivity of the MIP against analogous molecules, nine different OPPs (MCP, mevinphos, phosphamidon, omethoate, dimethoate, diazinon, phorate, parathion, fenitrothion) were selected to test the binding characteristic of MIP. A total of 1.0 mL of a mixture of 0.2 μg/mL of each organophosphorus was applied to the MIP and blank polymer cartridges, and then the compounds in both the washing and elution fractions were analyzed by GC/NPD. Fig. 5 showed the chromatograms of OPPs in standard solution, washing solutions, and elution fractions. It can be seen that almost all of the OPPs were completely removed from the blank column after the washing step. However, a different result was observed for the MISPE cartridge. MCP, the template molecular, was still totally retained on the MISPE column after the washing step. In addition, some OPPs such as mevinphos, phosphamidon and omethoate were also partly retained on the MISPE column. The recoveries of these compounds were higher than 80% except phosphamidon. The left OPPs cannot be recognized by the MIP and were completely separated from the target analytes. These results showed that the MIP exhibited highly selective binding affinity for MCP, mevinphos and omethoate and demonstrated that the adsorption of these OPPs was due to imprinted binding sites and not

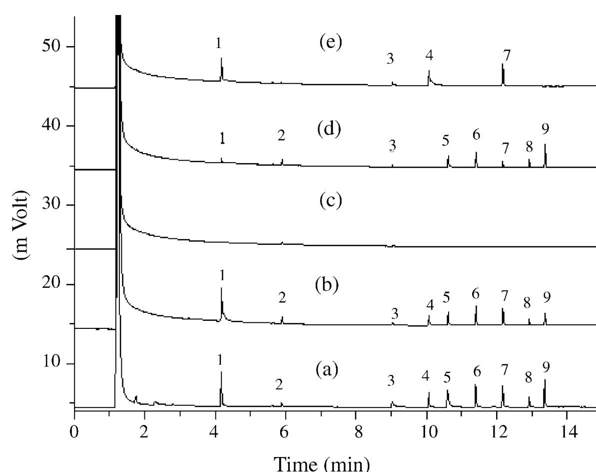

Fig. 5. Chromatograms obtained by off-line SPE of 1.0 mL of a mixture of 0.2 μg/mL of each OP: (a) standard solution; (b) blank polymer, washing fraction; (c) blank polymer, elution fraction; (d) MIP, washing fraction; (e) MIP, elution fraction. (1) Mevinphos, (2) phorate, (3) omathoate, (4) MCP, (5) dimethoate, (6) diazinon, (7) phosphamidon, (8) fenitrothion, and (9) parathion. Washing step: 2 mL of CH_2Cl_2/ACN (95:5, v/v); elution step: 2 mL of CH_2Cl_2/MeOH (90:10, v/v).

due to nonspecific binding, while other OPPs showed less or no binding. This could be easily explained by their close structural homology to MCP. From Fig. 1, it can be seen that there is only slight difference between the structure of MCP and those of mevinphos, phosphamidon, and omethoate. For mevinphos, the structural difference is only a –O instead of –N–H. For phosphamidon, 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. 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 [28]. The recovery of four selected OPPs (Table 3) showed that the MIP cartridge could be proved to be a powerful tool for the selective enrichment of four polar OPPs.

3.6. Determination of OPPs in spiked water samples

To demonstrate the applicability of reliability of this method for environmental application, real environmental water samples were selected and analyzed. Tap water and river water were spiked with the four polar OPPs at the

Table 3
Recoveries of four selected OPPs after loading of 1.0 mL of 0.2 μg/mL of each OPP onto the MIP cartridge ($n = 3$)

Analyte	Blank (% ± SD)		MIP (% ± SD)	
	Washing	Elution	Washing	Elution
Monocrotophos	100.1 ± 1.6	0	0	99.2 ± 1.7
Mevinphos	88.7 ± 2.9	0	12.6 ± 4.0	83.1 ± 2.3
Phosphamidon	101.2 ± 4.7	0	25.9 ± 2.6	72.4 ± 3.1
Omethoate	92.8 ± 4.9	0	9.4 ± 3.5	86.3 ± 1.5

100 ng/L concentration level and were preconcentrated by MISPE. The recoveries, reproducibility, and LOD of the method were calculated and summarized in Table 4. As can be seen, for analysis of four polar OPPs in the water samples, the analyte recoveries were higher than 80% except the phosphamidon. The relative standard deviation ($n = 3$) for quantitation was between 2.3% and 4.9% for tap water and between 2.6% and 5.5% for river water, which is a good value for real sample analysis. The limit of detection (LOD), defined here as the concentration for which a signal-to-noise ratio of three was obtained, was estimated from the chromatograms obtained from fortified water samples at 100 ng/L. LOD calculated for tap water was between 9 ng/L and 32 ng/L. LOD for river water sample was very similar and varied from 10 ng/L to 32 ng/L.

3.7. Determination of OPPs in spiked soil samples

The spiked soil sample was extracted according to section 2.8. Subsequently, the supernatant was decanted into a reservoir containing approximately 100 mL distilled water and performed using LLE or passed through the MISPE cartridge, the ENVI-18 cartridge. After the passage of the sample, the ENVI-18 cartridge was washed with 2 mL water and dried in a gentle stream of nitrogen. The analytes were then eluted with 10 mL of ACN. Solvent removal and residue reconstitution were the same as in the MISPE procedure. Fig. 6 showed the chromatograms of soil extracts after MISPE, ENVI-18 cartridge and dichloromethane LLE. It can be seen that an unknown compound could not be separated from MCP by LLE without an additional cleanup step. The recoveries, reproducibility, and LOD of the soil extracts were calculated and summarized in Table 5. It is clear that both LLE (65.8–85.2%) and ENVI-18 cartridges (51.5–82.4%) have poorer recoveries for four polar OPPs compared with MISPE (79.3–93.5%). Obviously, LLE has a very low selectivity and cannot remove interfere substances completely. The overlay of their peaks made the quantitation of MCP inaccurately. This was in agreement with [8]. SPE with ENVI-18 cartridge has also been shown to be limited to the handing of four polar OPPs. The mechanism of C_{18} bonded-phase extraction is based on non-polar interactions between the carbon–hydrogen bonds of the sorbent and the carbon–hydrogen of bonds of the analyte [29,30]. The pesticides under investigation are very polar and water soluble (as shown in Table 1), log K_{ow} values are below zero [9,31]. The obtained low recoveries were resulted from the early breakthrough of the analytes. However, the MISPE cartridge proved to be effective to separate and enrich four polar OPPs from soil extract. Moreover, the MIP proved to be very stable against high and low pH value, extreme pressure and temperature and favorable compatibility with organic solvents [32].

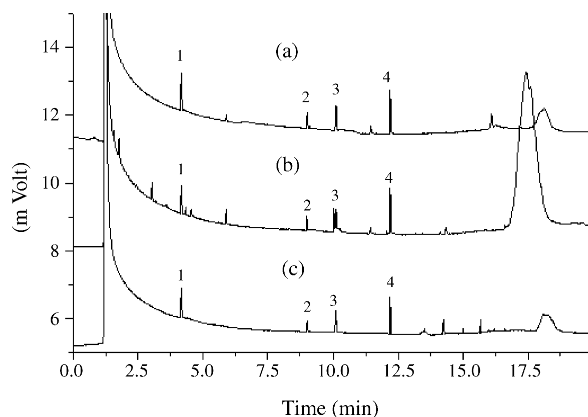

Fig. 6. GC/NPD chromatograms obtained by extracting OPPs from 5 g of soil spiked with 0.5 mL standard solution (1.0 μg/mL) on (a) MIP cartridge; (b) LLE and (c) ENVI-18 cartridge. (1) Mevinphos, (2) omathoate, (3) MCP and (4) phosphamidon. MIP cartridge—washing step: 2 mL of CH_2Cl_2/ACN (95:5, v/v), elution step: 2 mL of CH_2Cl_2/MeOH (90:10, v/v). ENVI-18 cartridge—washing step: 2 mL of water, elution step: 10 mL ACN.

Table 4
Recoveries (%), precision, and limits of detection (LOD) of OPPs after MISPE of water samples[a] (spiked at 100 ng/L)

Compounds	Tap water (1 L)			River water (1 L)		
	Recovery (%)	RSD	LOD (ng/L)	Recovery (%)	RSD	LOD (ng/L)
Monocrotophos	98.5	2.3	15	99.1	2.6	16
Mevinphos	81.3	3.6	9	82.4	5.2	10
Phosphamidon	79.6	4.1	12	77.5	3.9	12
Omethoate	86.1	4.9	32	84.4	5.5	32

LOD was defined as S/N = 3.
[a] RSD ($n = 3$).

3.8. Determination of OPPs in water and soil samples

The proposed method was applied to the analysis of six water samples and five soil samples collected from Yongan county (Fujian, China). None of the target analytes were detected in these water samples under the experimental conditions described. In soil samples, it was found that three OPPs except phosphamidon detected at levels in the range of 0.015–0.153 μg/g (as shown in Table 6). This confirmed the reliability and efficacy of the proposed method for the analysis of polar OPPs residues in real samples.

Table 5
Recoveries (%), precision, and limits of detection (LOD) of OPPs after MISPE of soil samples[a] (spiked at 100 μg/kg)

Compounds	MIP cartridge			LLE			ENVI-18 cartridge		
	Recovery (%)	RSD	LOD (μg/kg)	Recovery (%)	RSD	LOD (μg/kg)	Recovery (%)	RSD	LOD (μg/kg)
Monocrotophos	93.5	4.1	18	72.3	6.4	23	51.5	5.8	18
Mevinphos	82.9	5.6	12	79.5	4.1	14	82.4	4.3	12
Phosphamidon	79.3	3.9	16	85.2	5.2	19	72.3	5.4	16
Omethoate	85.8	5.8	34	65.8	7.9	42	63.5	7.6	35

LOD was defined as S/N = 3.
[a] RSD ($n = 3$).

Table 6
Residue levels of four polar organophosphorus pesticides (OPPs) (μg/g) of real soil samples[a]

Field	Monocrotophos (μg/g)	Mevinphos (μg/g)	Phosphamidon (μg/g)	Omethoate (μg/g)
1#	0.021 ± 0.002	0.015 ± 0.002	ND	ND
2#	ND	ND	ND	ND
3#	0.029 ± 0.003	ND	ND	0.153 ± 0.009
4#	ND	ND	ND	0.045 ± 0.003
5#	ND	0.026 ± 0.002	ND	ND

ND: not detected.
[a] Values are the mean of three replicates ± standard deviation.

4. Conclusion

In this work, MIPs selective for MCP were prepared and applied as the material for SPE in off-line separations. The MIP showed excellent affinity and selectivity to MCP and was therefore suitable for the application in SPE. The newly developed MISPE proved to be a powerful tool for the selective enrichment of four OPPs from water and soil samples. Its low cost of preparation and favorable compatibility with organic solvents allowed reliable, accurate analysis of the analytes within complex matrix at trace level. With optimized condition, the MISPE offered several practical advantages over other methods such as LLE and SPE with ENVI-18 materials. The presented approach demonstrated the application of MISPE for the analysis of OPPs from real environmental samples for the first time and revealed a substantial potential of this advanced approach soon.

Acknowledgement

This research work has been supported by the National Natural Science Foundation of China (No. 20405013).

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