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**Abstract.** Molecular imprinting technique offers a means of producing practical materials that are able to recognize a certain molecule in terms of shape, size and chemical functionality. In order to obtain a highly selective recognition of organophosphorus pesticides (OPPs), we synthesized molecularly imprinted polymers (MIPs) using pirimiphos-methyl as the template, methacrylic acid as the monomer and ethylene glycol dimethacrylate as the crosslinker. After polymerization, molecularly imprinted solid-phase extraction (MISPE) was used for the selective preconcentration of OPPs. The preparation methods and synthesis conditions of MIPs were discussed, and the specificity of MIPs and nonimprinted polymers were investigated. The results showed that MIPs enable the selective extraction of pirimiphos-methyl successfully from water sample, and demonstrated the potential of MISPE for selective and cost-effective sample pretreatment.

**Keywords:** Molecularly imprinted polymers, Solid phase extraction, Organophosphorus pesticides

## 1 Introduction

Organophosphorus pesticides (OPPs) have often been employed in farmland cultivation over the last several decades and are still continuously used in modern agricultural systems[1]. These OPPs exhibit acute or chronic toxicity to human, environment and the biota thus emphasizing the need for efficient analytical procedures to monitor potential risks. Most OPPs are easily analyzed by GC and HPLC. Generally, the trace analysis needs a pretreatment step in order to reduce the matrix interference and enrich the analyte. This is often performed by solid-phase extraction (SPE)[2-4].

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Molecular imprinting is a versatile technique that creates molecular assemblies of desired chemical structures and properties[5]. During last decade, molecularly imprinted polymers (MIPs) have demonstrated a great potential as selective sorbents and have been widely used for the clean-up of samples in SPE processes[6-8], namely molecularly imprinted solid-phase extraction (MISPE). The ongoing research has proven that MIPs can be efficiently used in this field[9-10].

In this article, we reported the synthesized method of acrylic-based MIPs following the non-covalent approach and the selective use of MISPE technique for the analysis of organophosphorus pesticides.

## 2 Materials and methods

### 2.1 Materials

Pirimiphos-methyl (99.7%), methylnitrophos (99.2%) and malathion (97.2%) were purchased from J＆K Scientific (Beijing, China). Methacrylic acid (MAA) and ethylene glycol dimethacrylate (EGDMA) were purchased from Sigma. The initiator 2,2'-azobisisobutyronitrile (AIBN) was purchased from Shanghai Chemical Plant (Shanghai, China). All solvents of analytical grade were purchased from Beijing Chemical Reagent (Beijing, China). All syntheses were carried out using distilled-deionized water (18.2 MΩ.cm, PALL system). All solutions were filtered through a 0.45μm membrane from Millipore before use.

### 2.2 Polymer preparation

The template pirimiphos-methyl (0.305g, 1mM) and functional monomer MAA (0.344g, 4mM) were dissolved in 5.6mL dichloromethane in a 40-mL glass tube with slightly shaking for 6h. To this solution, cross-linker EGDMA (3.964g, 20mM) and AIBN (40mg, 0.24mM) were added in steps. Then, the mixture was deoxygenated with nitrogen for 15min, followed by degasification under vacuum for 5min and sealed. The polymerization was carried out by heating the mixture in a 60℃ water bath for 36h. The obtained polymer was ground to fine powders and sieved to obtain 60-70μm particles. These particles were extracted in a Soxhlet for 24h with methanol-acetic acid (9:1, v/v) until no residue of template was found in the rinses.

The corresponding non-imprinted polymers (NIPs) for comparison experiments were prepared in the same manner but without addition of template.

### 2.3 MISPE protocol

Two hundred milligrams of the cleaned-up MIPs (or NIPs) were put into a 10mL vial and incubated with methanol, standing at ambient temperature with occasional shaking for 24h. Then the slurry was transferred into a 3mL polypropylene SPE

cartridge and stood for 30min. After that, polyethylene frit was carefully put onto the polymer to stabilize the sorbents. MIPs of the size 60-70μm proved to be an acceptable compromise between homogeneity and permeability of SPE cartridge. Prior to use, the MIPs (or NIPs) SPE-cartridges were conditioned by washing with 10mL methanol-acetic acid (9:1, v/v) and 2mL methanol, followed by 2mL water. For the MISPE process, standard solution (a mixture of pirimiphos-methyl, methylnitrophos and malathion, 1μg/mL) and a spiked water sample were loaded onto the MISPE cartridges at a flow rate of 0.4mL/min respectively. And then the SPE cartridges were washed with 2mL dichloromethane/acetonitrile (95:5, v/v) and eluted with 2mL dichloromethane/methanol (90:10, v/v) by steps. The eluate was immediately dried under a stream of nitrogen, and the residue was dissolved in 1mL dichloromethane for GC-MS analysis.

### 2.4 GC-MS assay

The assay was conducted by Shimadzu GC-MS QP2010 Plus. The analyses were carried out on a gas chromatograph fitted with a HP-5 MS capillary column (30 m×0.25mm id; 0.25μm film thickness). Analytical gas chromatography conditions were as follows: injector temperature 230℃; oven temperature held at 120℃ for 5 min, then programmed to increase from 120℃ to 150℃ at a rate of 5℃/min and held for 7min; carrier gas, helium at a flow rate of 1mL/min; Mass spectrometer conditions: ionization mode with EI, electron energy 70eV, ion source temperature 230℃, interface temperature 220℃. Instrument operation and data processing was done through the LabSolutions (version 2.50) software.

## 3 Results and discussion

### 3.1 Synthesis of polymer

The choice of polymerisation solvent is the key point of the adduct formation and the promotion of the imprinting efficiency[11]. Dichloromethane is one of the most widely used solvents, since it satisfactorily dissolves all the reaction components and does not suppresses hydrogen bonding. We speculate that the use of dichloromethane can enhance the unspecific binding of analyte to the crosslinker. In addition, the nature of the crosslinker is another key factor of the polymer specificity[12]. The reactivity of the crosslinker should be similar to that of the functional monomer. And the mole ratios of crosslinker to functional monomer are also important[13]. Generally, hydrogen bonding is dependent on both distance and direction between monomers and templates. EGDMA as the shortest crosslinker led to the highest selectivity in the polymer[14].

### 3.2 Specificity of polymer

One merit of MISPE is that the polymer sorbents have good selectivity for the template molecule. To evaluate the specificity of this kind of SPE materials, the molecular recognition properties of three different OPPs (pirimiphos-methyl, methylnitrophos, malathion) was investigated. A total of 1.0mL of a mixture of 1μg/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-MS.

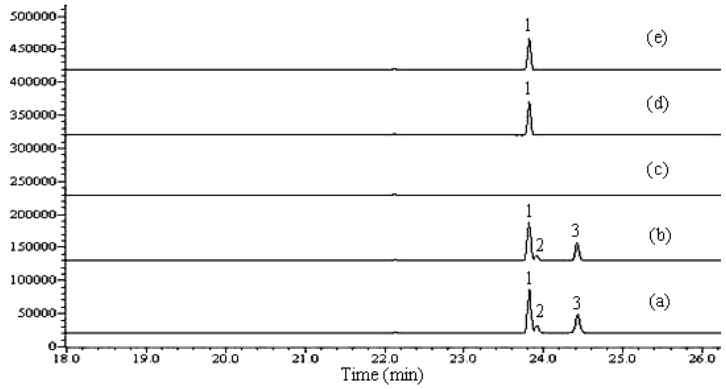


**Fig. 1.** Chromatograms obtained by off-line SPE of 1.0mL of a mixture of 1μg/mL of each OPP. (a) standard solution; (b) NIP, washing fraction; (c) NIP, elution fraction; (d) MIP, elution fraction; (e) water sample. (1) pirimiphos-methyl; (2) methylnitrophos; (3) malathion. Wash step: 2mL dichloromethane/acetonitrile (95:5, v/v). Elution step: 2mL dichloromethane/methanol (90:10, v/v).

Fig.1 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. Pirimiphos-methyl, the template molecular, was still totally retained on the MISPE column after the washing step. The recovery of pirimiphos-methyl was higher than 80%. In addition, the left OPPs were not retained on the MISPE column. In other words, they cannot be recognized by the MIPs and were completely separated from the target analyte. These results showed that the MIP exhibited highly selective binding affinity for pirimiphos-methyl and no binding for the left OPPs. Although there is only slight difference between the structures of those three OPPs, this further explains that the imprinting is not only based on the interaction of the functional groups of the analyte but also based on the combined effect of shape and size complementarily[15]. The recovery of three OPPs (Table 1) showed that the MIP cartridge could be proved to be a powerful tool for the enrichment of pirimiphos-methyl.

### 3.3 Determination of pirimiphos-methyl in spiked water sample

To demonstrate the applicability of reliability of this method for environmental application, real environmental water sample was selected and analyzed. Tap water was spiked with pirimiphos-methyl at the 1μg/mL concentration level and was preconcentrated by MISPE. The recovery and reproducibility of this method were calculated and summarized in Table 1. As expected, for analysis of pirimiphos-methyl in the water sample, the analyte recovery was higher than 80%.

**Table 1.** Recoveries of three OPPs after loading of 1.0mL of 1μg/mL of each OPP onto the SPE cartridges (n=3)

| Analyte | NIP(%±SD) | | MIP(%±SD) | |
|---|---|---|---|---|
| | Washing | Elution | Elution | Tap water |
| Pirimiphos-methyl | 97.6±2.5 | 0 | 83.2±3.1 | 80.1±2.3 |
| Methylnitrophos | 96.1±3.9 | 0 | 0 | 0 |
| Malathion | 99.3±2.6 | 0 | 0 | 0 |

## 4 Conclusion

In this report, we discussed the utility of molecular imprinting technology for the organophosphorus pesticides detection. MIPs selective for pirimiphos-methyl was prepared and applied as the material for SPE in off-line separations. The polymer showed well affinity and selectivity to pirimiphos-methyl. And the MISPE proved to be an effective tool for the enrichment of pirimiphos-methyl from water sample. For organophosphorus pesticides, the MISPE approach provided simpler methodology and significant increases in selectivity relative to the conventional methods. And we feel that this approach will be a useful analytical tool for analyzing complex samples, especially in performing the initial screening of libraries against poorly characterized receptors.

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