



Molecularly imprinted polymer coated on stainless steel fiber for solid-phase microextraction of chloroacetanilide herbicides in soybean and corn

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ABSTRACT

A molecularly imprinted polymer (MIP) with metolachlor as template was firstly coated on stainless steel fiber through chemical bonding strategy to solve the fragility problem of silica fiber substrate for solid-phase microextraction. The surface pretreatment of stainless steel fiber and the polymerization conditions were investigated systematically to enhance the preparation feasibility and MIP coating performance, and then a porous and highly cross-linked MIP coating with 14.8- μm thickness was obtained with over 200 times re-usability which was supported by non-fragile stainless steel fiber adoption. The MIP coating possessed specific selectivities to metolachlor, its metabolites and other chloroacetanilide herbicides with the factors of 1.1–4.6. Good extraction capacities of metolachlor, propisochlor and butachlor were found with MIP coating under quick adsorption and desorption kinetics, and the detection limits of 3.0, 9.6 and 38 $\mu\text{g L}^{-1}$ were achieved, respectively. Moreover, the MIP-coated stainless steel fiber was evaluated for trace metolachlor, propisochlor and butachlor extraction in the spiked soybean and corn samples, and the enrichment factors of 54–60, 27–31 and 15–20 were obtained, respectively.

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1. Introduction

Solid-phase microextraction (SPME), pioneered by Arthur and Pawliszyn [1], is a simple, time-saving, solvent-free and powerful pretreatment technique for the separation and enrichment of various organic compounds from liquid, solid or gaseous samples at trace level. SPME is based on the partitioning of compounds between sample and the coating on a silica fiber, so the coating investigation always takes a crucial role in SPME development, and lots of materials, such as polyimide [1], polydimethylsiloxane (PDMS) [2], polyacrylate (PA) [3], carbowax/divinylbenzene (CW/DVB) [4], carbopack [5] and polypyrrole [6], were adopted to achieve higher applicabilities for different kinds of compounds. However, the selectivities of these coatings were insufficient. Accordingly, some new coatings with enhanced selectivities were reported recently, such as fullerene [7,8], crown ester [9–11], calixarene [12] and β -cyclodextrin [13].

Acclaimed as the artificial antibody, molecularly imprinted polymers (MIPs) possesses predetermined selectivities to template molecules and is characterized with high chemical and physical stabilities, good preparation convenience and long lifetime. Owing to

these, MIPs was applied as the recognition or separation materials in various fields, such as chromatography [14,15], solid-phase extraction [16,17], chemical sensor [18,19] and immunoassay [20]. Recently, the application of MIP in SPME for sample pretreatment was attractive and prospective [21–28]. With improved selectivities, the matrix interferences in complicated samples could be reduced with the MIP-coated SPME fibers. In our previous works [29–31], MIP-coated fibers could be used for over 80 times as a result of the chemical bonding between MIP and the silica fiber. However, caused by the fragility of silica fiber [1], their real re-usabilities were frequently restricted [29–31]. As we know, in contrast with coating, few improvements have been made for the substrate material of SPME fibers. Since the emergence of SPME technique, silica fiber was always applied due to its characteristics of good toughness and chemical modification convenience. Whereas, the lifetime of SPME fiber was frequently shortened by the breaking of fragile silica fiber [32], no matter what coating was used. Although the commercial SPME fibers could be protected with a hollow stainless steel tube when the fibers were not in use, the breaking incidents would still occurred occasionally [33].

With the characteristics of good mechanical properties and easy acquirement, flexible and non-fragile stainless steel fibers are suitable as the substrate of SPME coating to overcome above disadvantages. As a matter of fact, stainless steel is resistant to corrosion in various environments such as chloride mediums, strongly

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acidic solutions and alkaline conditions [34]. Consequently, stainless steel was applied extensively in numerous fields. Moreover, it could be coated with various materials such as Al_2O_3 [35], TiO_2 [36], polypyrrole [37] and graphite modified polypyrrole [38] for further applications. Recently, Yan's group reported the coating of metal-organic framework 199 films on stainless steel SPME fibers by in situ hydrothermal growth for gaseous benzene detection [39]. It was expected that the adoption of MIP-coated stainless steel fiber would be beneficial to further SPME developments such as shape-tailored application and in vivo assay. However, probably caused by the difficulties in surface treatment and chemical modification, little attention has been focused on the coating of MIP material on stainless steel surface. Lai's group reported MIP preparation by electropolymerization of pyrrole onto a stainless steel frit [37], but stainless steel frit was only used as working electrode and MIP sustainment.

In this paper, MIP with metolachlor as template was prepared and firstly coated on the surface of stainless steel fiber through chemical bonding strategy. The pretreatment of stainless steel fiber was investigated, and the preparation conditions of MIP coating were studied through two-stage optimizations. Subsequently, the coating characterization, extraction condition optimizations and spiked plant sample analysis were performed for the evaluation of MIP-coated stainless steel fibers.

2. Experimental

2.1. Chemicals and reagents

Metolachlor, propisochlor, acetochlor and butachlor were obtained from Kesai Chemical Industry Corporation (Jinan, China). Alachlor and pretilachlor were obtained from Sigma-Aldrich (St. Louis, MO, USA). Hydroxymetolachlor, deschlorometolachlor and desmethylmetolachlor were kindly provided by Shenzhen Bureau of Quality and Technical Supervision (Shenzhen, China). Acrylamide (AA), 4-vinylpyridine (4-VP), trimethylolpropane trimethacrylate (TRIM) and ethylene glycol dimethacrylate (EGDMA) were purchased from Sigma-Aldrich. Methacrylic acid (MAA) and azo(bis)-isobutyronitrile (AIBN) were purchased from Damao Reagent Plant (Tianjin, China). Vinyltriethoxysilane (VTEOS) was purchased from Shengda Fine Chemical Industry Corporation (Beijing, China). The HPLC-grade methanol and acetonitrile were purchased from Merck (Darmstadt, Germany). Water was double distilled. All other reagents were of analytical grade. All solutions used for HPLC mobile phase were filtered through a 0.45- μm nylon filter. The stainless steel fibers (316L, 200 μm O.D.) were obtained from an ironware factory. The commercial SPME fibers with PA, PDMS and PDMS/DVB coatings were purchased from Supelco (Bellefonte, PA, USA). Alkaline oil-removing solution was prepared with sodium hydroxide of 50 g L^{-1} , sodium carbonate of 20 g L^{-1} and sodium phosphate tribasic of 20 g L^{-1} in water. Oxidation solution I was prepared with sodium nitrite of 50 g L^{-1} and sodium carbonate of 10 g L^{-1} in water. Oxidation solution II was 5% hydrogen peroxide. Oxidation solution III was prepared with potassium dichromate of 20 g L^{-1} and sodium carbonate of 2 g L^{-1} in water. Oxidation solution IV was sulfuric acid of 0.1 mol L^{-1} . For solutions I–III, oxidation was performed at 60 °C for 30 min, and for solution IV, oxidation was performed at room temperature for 30 min.

2.2. Surface treatment of stainless steel fiber

The stainless steel fibers were cut to the length of 6.0 cm. Fiber cleaning was performed in acetone for 30 min and then in alkaline oil-removing solution at 80 °C for 20 min. Following fiber oxida-

tion was performed with oxidation solution I at 60 °C for 30 min. After that, the fibers were rinsed 3 times with methanol, immersed into vinyltriethoxysilane–water–methanol (1:8:1, v/v/v) solution for 30-min silylation, and then dried in an oven at 150 °C for 120 min. Finally, these fibers were rinsed 3 times with ethanol and dried with a stream of nitrogen.

2.3. Preparation of metolachlor MIP-coated stainless steel fiber

Metolachlor MIP used in this paper was developed in our laboratory. 13 μL of metolachlor and 17 μL of MAA were added into 2.5 mL of toluene for 12 h swirling in an oscillator at 5 °C, and then 3.9 mg of AIBN and 255 μL of TRIM were added and mixed adequately. 2 mL of above polymerization solution was transferred into a small glass tube and deoxygenized with nitrogen gas for 5 min. Subsequently, a silylated stainless steel fiber was inserted, and the tube was sealed immediately to perform the polymerization at 60 °C in a thermostatic water bath (Senxin, Shanghai, China). Three hours later, the fiber was pulled out from the tube, and a thin layer of MIP coating was observed on the fiber surface. Then the fiber was immersed again into another fresh polymerization solution, and then it was coated repeatedly through identical procedures mentioned above. The total repeated coating times of 10 was applied. Finally, the fiber was repeatedly soaked in 10-mL 10% (v/v) acetic acid solution in methanol for 30 min to remove template molecules, until metolachlor could not be detected by HPLC in the soaking solution. The obtained MIP coating was scraped from the fiber top to obtain a uniform length of 10 mm. Non-imprinted polymer (NIP)-coated stainless steel fiber was prepared simultaneously following the identical procedures except the addition of metolachlor template.

For the investigation of preparation conditions, the uniformity and surface morphology evaluation of MIP coatings was performed with a 50iPOL polarizing microscope (Nikon, Japan) under the magnifications of 200 and 400. The coating thickness was measured with a scale eyepiece which was calibrated through a 0.01 mm slide micrometer. The difference in fiber diameter between naked and MIP-coated fibers was used to calculate the value of MIP coating thickness.

2.4. Coating characterization

The scanning electron micrography was obtained with an XL-30 scanning electron microscope (Philips, Eindhoven, Netherlands). The infrared absorption spectrum of MIP coating was obtained in an IR-prespige-21 FT-IR spectrometer (Shimadzu, Japan). The thermogravimetric analysis over 50–600 °C was performed in an STA-409 PC thermogravimetric analyzer (Netzsch, Selb/Bavaria, Germany) with the heating rate of 10 °C min^{-1} .

2.5. SPME procedures

The standard solution or sample solution was added into a 5-mL glass vial and dried with a stream of nitrogen, and then 3-mL extraction solvent of hexane and a magneton were added. A MIP- or NIP-coated stainless steel fiber was immersed into the solution for 30-min extraction at the stirring rate of 750 rpm. Subsequently, the fiber was pulled out and rinsed with hexane for 10 s, and then it was immersed into an SPME–HPLC coupling device (Supelco, Bellefonte, PA, USA) for 10-min desorption with about 60- μL methanol. After desorption, the fiber was pulled out and all desorption solution was injected into chromatographic column for analysis, and then the fiber was immersed into methanol for 10-min condition to be ready for the next extraction. For the adsorption kinetics investigation of MIP coating, desorption time of 10 min was applied, and for desorption study, extraction time of 30 min was applied.

As comparison, the commercial SPME fibers with PA, PDMS, or PDMS/DVB coating were applied for spiking sample analysis. Referred to the reported conditions [40], SPME was carried out in 3-mL aqueous solution for 60 min at the stirring speed of 750 rpm, and the desorption was carried out with acetonitrile/water (55:45, v/v, mobile phase) in the SPME–HPLC coupling device for 10 min.

2.6. Chromatographic system

The chromatographic analysis was performed with a LC-10ATvp HPLC (Shimadzu, Japan), an ultraviolet detector and an SPME–HPLC coupling device. The chromatographic column was Dikma C₁₈ (250 mm × 4.60 mm I.D., 5 μm packing, Beijing, China). The mobile phase was used at a constant flow rate of 1.0 mL min⁻¹. Elution was performed with a gradient of acetonitrile and water, in which the acetonitrile content was decreased linearly from 55 to 50% (v/v) during 2 min, from 50 to 40% (v/v) during 18 min, from 40 to 0% (v/v) during 20 min and held at 0% for 10 min, and then it was adjusted back to 55% (v/v) and held for 5 min. Detection wavelength was 197 nm. The quantification of chloroacetanilide herbicides, metolachlor metabolites and toluene was carried out by the peak area measurement using external standard calibration. Restricted by the difficulty in chromatographic separation, only metolachlor, propisochlor and butachlor were selected for extraction performance and real sample analysis investigations.

2.7. Sample pretreatment

Soybean and corn, frequent exerting objects of chloroacetanilide herbicides, were selected for spiking sample analysis. The dry soybean and corn were ground, and then sieved with a mesh gauge. 2 g of soybean or corn powder with the particle size of 0.198–0.246 mm was mixed with 0.04, 0.2 or 1.0 mL of 10.0 mg L⁻¹ metolachlor, propisochlor and butachlor mixed standard solution in acetonitrile, and then was treated with ultrasonic extraction in 30-mL acetonitrile for 10 min. The extraction solutions were filtrated and concentrated with nitrogen gas, and then were quantified to the volume of 20 mL with acetonitrile. The spiking concentrations of soybean and corn solutions were obtained with three levels of 20.0, 100 and 500 μg L⁻¹.

3. Result and discussion

3.1. Surface treatment of stainless steel fiber

The simplified scheme of surface treatment of stainless steel fiber is illustrated in Fig. 1S(a) in the supplemental material.

When purchased, the surface of straightened stainless steel fibers was covered with lubricating oil, no MIP could be coated on these oily stainless steel fibers. So the fibers were rinsed with organic solvents such as acetone or ethanol to remove oil, but the effect was dissatisfactory. Consequently, after rinsed with acetone for 1 h, the fibers were treated with alkaline oil-removing solution for 30 min at 80 °C to ensure the complete removing of lubricating oil.

After the oil removing, the surface of stainless steel fiber was oxidized and functionalized with hydroxyl which was frequently used as the active group on silica materials to bond with silane reagent [41,42]. Four frequently used oxidation solutions I–IV based on sodium nitrite, hydrogen peroxide, potassium dichromate and sulfuric acid were investigated. The results indicated that good coating uniformity and compactness were obtained only with oxidation solutions I and IV, and the coating thicknesses were 1.7 and 1.1 μm, respectively. The non-oxidized stainless steel fiber was also used for MIP coating preparation, and the results of 1.1-μm coating thickness, poor coating uniformity and coating breaking were

mainly caused by the physical deposition of MIP coating on fiber surface. In contrast, MIP coating could be chemically bonded on the surface of oxidized stainless steel fiber through the bridging of metal-hydroxyl and silane, and this would ensure the coating re-usage over 200 times without remarkable morphology changes. The oxidation solution I was applied through the following investigations.

The fiber silylation was investigated with VTEOS–ethanol (v/v 1:9) and VTEOS–water–ethanol (v/v/v 1:1:8) solutions, and the MIP coating thicknesses of 1.2 and 1.6 μm were obtained, respectively. In contrast, when non-silylated stainless steel fiber was used, the coating thickness was only 0.8 μm. Moreover, as a result of weak physical deposition, the coating was easily broken off. It was demonstrated that VTEOS silane with vinyl group acted the bridge between organic polymer and inorganic metal to ensure MIP coating firm chemical bonding and adequate thickness. The silylation solution of VTEOS–water–ethanol was applied through the following investigations.

3.2. Preparation of MIP coating

The simplified scheme of MIP coating preparation is shown in Fig. 1S(b) in the supplemental material. It was well-known that the conditions such as solvent, monomer, cross-linker and component proportions were important for MIP preparation feasibility and capability, but previous studies for optimization were leaned solely to morphology character [29–31] or extraction performance [22–24]. With these facts, the polymerization conditions were studied through two-stage optimizations in this paper. Firstly, above conditions were optimized respectively with coating thickness, uniformity and compactness as the rules. Subsequently, two or three preferred selections (see Table 1) for each condition were applied for MIP-1 to MIP-9 preparation respectively on stainless steel fiber with the repeated coating times of five, and then second optimization was performed with the rules of extraction capacity and selectivity. As shown in Table 1, the performance of MIP coating was affected markedly by the preparation conditions. Low extraction amounts were found with MIP-7 and MIP-8, and poor selectivities were found with MIP-5 to MIP-7. It was indicated that monomer, cross-linker and component proportions played important roles in MIP preparation. The conditions for MIP-1 were proved to be optimal because of the obtained highest extraction amount of 0.55 ng and the best selectivity coefficient (defined as the ratio of extraction amount of metolachlor with MIP to that with NIP coating) of 5.5. Through two-stage optimization strategy, both morphologic structure and extraction performance of MIP coating could be improved.

However, when MIP coating was synthesized with the optimal conditions and coated on the stainless steel fiber for only 1 time, the obtained thickness was about 2.0 μm and it was too thin to achieve satisfactory binding capacity. Moreover, the increase of polymerization time could not improve the thickness of MIP coating markedly, and the prolonged time would result in the enhancement of cross-linking degree and subsequent difficulty in pulling the fiber out of solid polymer. This thickness problem could be solved with the multiple bulk co-polymerization method, in which the same coating procedures were performed repeatedly on one stainless steel fiber. The results demonstrated that MIP coating thickness was increased with the repeated coating times, and a good linearity was observed with the correlation coefficient of 0.9986, as shown in Fig. 2S in the supplemental material. One addition in the repeated coating times would result in the thickness increase of about 1.2 μm. Simultaneously, RSD of thickness was reduced from 16% to 2.6% when repeated coating times arrived at 10, and the batch to batch repeatability was good with the thickness difference of only 0.2 μm. Whereas, further coating operation would result in the decline of preparation repeatability.

Table 1
Optimization of polymerization conditions for metolachlor MIP coating preparation.

	Solvent	Monomer	Cross-linker	Volume ratio of monomer plus cross-linker to solvent	Molar ratio of template to monomer	Extraction ^a amount with MIP coating (ng)	Extraction amount with corresponding NIP coating (ng)	Selectivity factor ^b
MIP-1	Toluene	MAA	TRIM	1:9	1:4	0.55	0.10	5.5
MIP-2	Acetonitrile	MAA	TRIM	1:9	1:4	0.40	0.13	3.1
MIP-3	Benzene	MAA	TRIM	1:9	1:4	0.33	0.12	2.8
MIP-4	Toluene	4-VP	TRIM	1:9	1:4	0.22	0.10	2.2
MIP-5	Toluene	AA	TRIM	1:9	1:4	0.33	0.33	1.0
MIP-6	Toluene	MAA	EGDMA	1:9	1:4	0.34	0.24	1.4
MIP-7	Toluene	MAA	TRIM	1:6	1:4	0.10	0.14	0.7
MIP-8	Toluene	MAA	TRIM	1:9	1:1	0.19	0.10	1.9
MIP-9	Toluene	MAA	TRIM	1:9	1:8	0.38	0.10	3.8

^a Extraction conditions: 670 $\mu\text{g L}^{-1}$ metolachlor in hexane of 0.15 mL was extracted with MIP- or NIP-coated stainless steel fiber for 120 min, desorption was performed in methanol of 0.15 mL for 30 min, injection volume for HPLC analysis was 10 μL .

^b Selectivity factor was defined as the ratio of extraction amount of metolachlor with MIP coating to that with corresponding NIP coating.

When repeated coating times added up to 12, the RSD of thickness increased to 6.1%. This was probably because that increased thickness would deepen the accumulated impact of polymerization condition variation along with the repeated coating procedures. Moreover, the coating compaction in aging and the swelling in template elution were enhanced, and then the coating cracking was observed markedly on the fiber surface.

To optimize the repeated coating times selection, coating lifetime investigation was performed. MIP-coated stainless steel fibers with the repeated coating times of 1–12 were applied simultaneously for 200 repetitious SPME operations, including 30-min extraction in hexane under the stirring speed of 750 rpm and subsequent 10-min desorption in methanol without stirring. The lifetime was counted till the falling or breaking of MIP coating on fiber surface. The micrographs of intact and breaking MIP-coated stainless steel fibers after 200 extractions and naked fiber were shown in Fig. 4S in the supplemental material. Apparently, the surface of naked stainless steel fibers is extremely rugged and this would be helpful to the stability of MIP coating. Moreover, rubbing abrasion was not observed on all fiber surfaces, but for some fibers, the MIP coatings were dropped off massively. Consequently, it could be concluded that the lifespan of MIP coating was affected mainly by its swelling in extraction and desorption solvents. Because the coating swelling was affected markedly by the thickness and thin layer of MIP coating was advantageous to its stability [29–31], the MIP coatings with repeated coating times of 1–4 were more stable and their lifetimes were longer, as shown in Fig. 3S in the supplemental material. After coating times of 4, lifetime decreased. However, there was a peak variation around 10 which could not be explained and would be studied in further research. Among 12 fibers, three fibers with repeated coating times of 2, 4 and 10 could keep MIP coating intact after 200 extractions, which were much longer than the lifetime of up to 100 for commercial coatings on silica fibers. Considering the coating thickness and lifetime, the repeated coating times of 10 was selected, and the coating thickness of finally prepared MIP-coated stainless steel fiber was 14.8 μm with RSD of 2.8%. Compared with the commercial or other home-made SPME fibers, the improvements of over 200 re-use times and non-fragile stainless steel substrate made MIP-coated stainless steel fiber a promising and practical tool for sample pretreatment.

3.3. Morphology, structure and stability investigations

The scanning electron micrograph of MIP-coated stainless steel fiber under the magnification of 10,000 \times is shown in Fig. 1. Highly cross-linked and porous morphological structure was observed. Compared with the commercial PDMS, PA and PDMS/DVB coatings with thickness of 60–100 μm , 14.8- μm MIP coating was disadvantageous to the extraction capacity. However, its special porous

structure would be beneficial to quick adsorption and desorption of analytes, and could provide sufficient and accessible recognition cavities for the extraction of trace metolachlor in real samples.

The infrared spectra investigation was performed to validate the chemical structure of metolachlor MIP coating. Four strong infrared absorption peaks of 3495, 2971, 1732 and 1468 cm^{-1} were found, which were attributed to hydroxyl groups, methyl groups, carbonyl groups and methyl groups, respectively. Simultaneously, a minor peak around 1640 cm^{-1} indicated the presence of residual C=C bonds in MIP coating. These characteristic groups were consistent with MAA monomer and TRIM cross-linker used in metolachlor MIP coating preparation shown in Fig. 1S(b).

The thermogravimetric analysis was applied to investigate the thermal stability of MIP coating. The results indicated that an obvious mass loss occurred at around 280 $^{\circ}\text{C}$ and the fastest mass loss occurred at 468 $^{\circ}\text{C}$. The chemical stability was studied with the soaking test, in which MIP-coated stainless steel fibers were immersed into water, methanol, acetonitrile, acetone, chloroform, ethyl acetate, benzene, toluene and 10% (v/v) acetic acid in methanol for 24 h, respectively. After that, these fibers were observed with microscope and were used to extract 0.10 mg L^{-1} metolachlor standard solution. The results indicated that all MIP coatings retained good surface quality and extraction performance.

3.4. Coating selectivity

For the selectivity evaluation of MIP coating, chloroacetanilide herbicides (metolachlor, acetochlor, alachlor, propisochlor, preti-

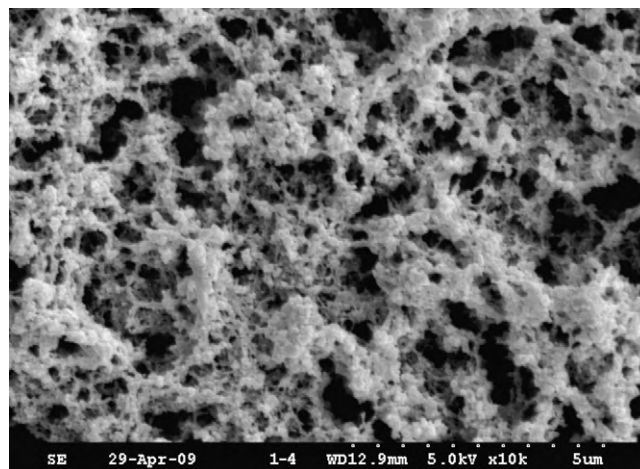


Fig. 1. Scanning electron micrograph of metolachlor MIP-coated stainless steel fiber with magnification of 10,000 \times .

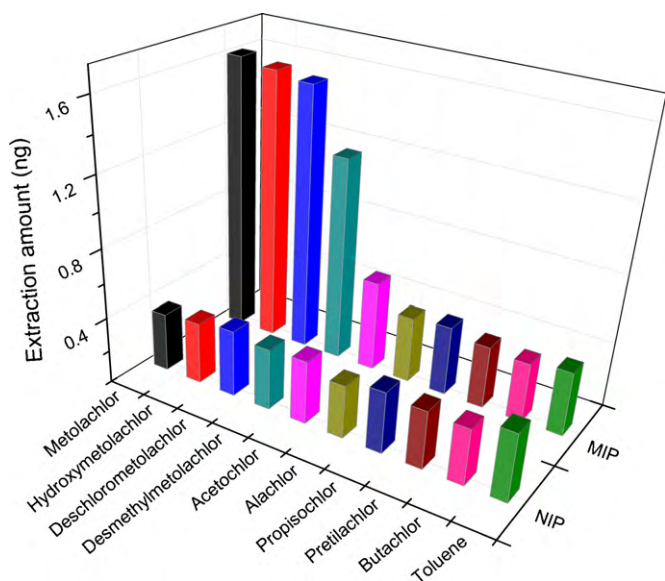


Fig. 2. Extraction amounts of metolachlor, hydroxymetolachlor, deschlorometolachlor, desmethylmetolachlor, acetochlor, alachlor, propisochlor, pretilachlor, butachlor and toluene with MIP or NIP coating at $100 \mu\text{g L}^{-1}$ level.

lachlor and butachlor), metolachlor metabolites (hydroxymetolachlor, deschlorometolachlor and desmethylmetolachlor) and reference compound of toluene were selected as the target and NIP coating as the comparison. The solutions of these compounds were prepared individually with the concentration of $100 \mu\text{g L}^{-1}$ to avoid the competitive adsorption. According to the results shown in Fig. 2, MIP coating possessed specific selectivities to metolachlor, hydroxymetolachlor, deschlorometolachlor and desmethylmetolachlor with the high selectivity factors of 4.60, 4.27, 3.88 and 3.24, respectively. In contrast, weaker selectivities were found to acetochlor, propisochlor, alachlor, butachlor and pretilachlor with the factors of 1.42, 1.25, 1.14, 1.09 and 1.06, respectively. This was mainly caused by the structure differences between target molecule and metolachlor template, as shown in Fig. 3. Clearly, the differences consisted in functional oxyalkyl chain and chloroacetyl substitution on amino group, whereas the oxyalkyl chain should

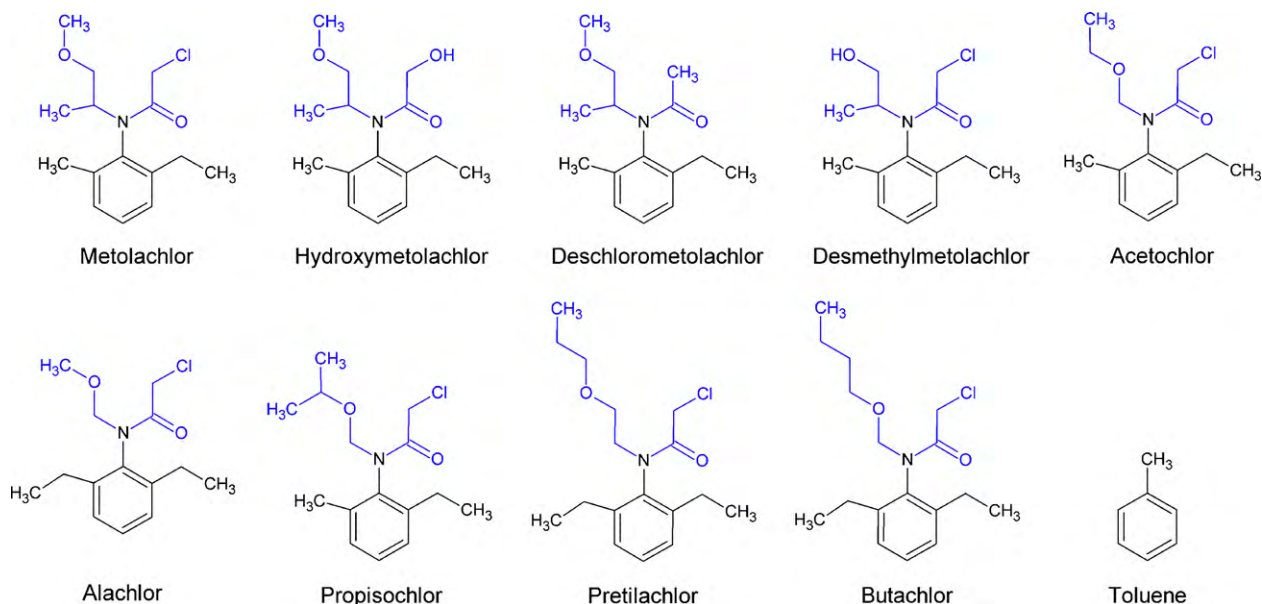


Fig. 3. Chemical structures of metolachlor, metolachlor metabolites, other chloroacetanilide herbicides and reference compound.

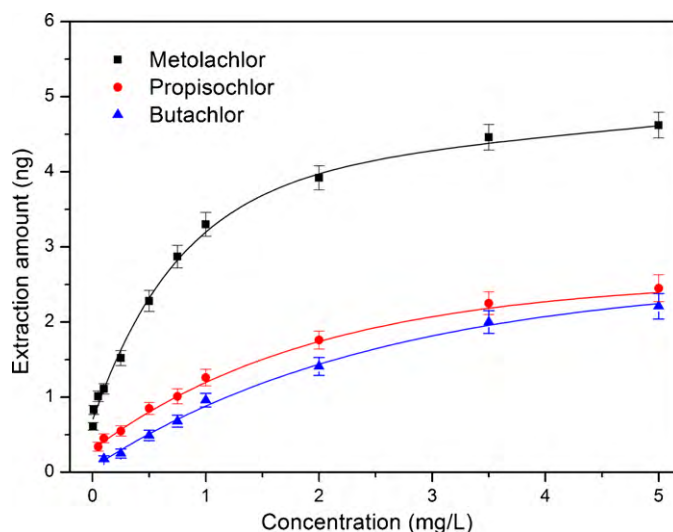


Fig. 4. Extraction amount curves of metolachlor MIP coating to metolachlor, propisochlor and butachlor mixed standard solutions of $0.0050\text{--}5.0 \text{ mg L}^{-1}$.

be the essential imprinting factor due to its higher accessibility of hydrogen-bond recognition sites and larger size for space-complementarity recognition. This could be proved by the specific selectivities found for three metabolites which possessed similar oxyalkyl chain in size and oxygen position with metolachlor, but for other chloroacetanilide herbicides, the recognition was affected by the structure differences in size, length and oxygen position of oxyalkyl chain. For toluene, there were no similarities in size, shape and functional groups with metolachlor, so the extraction was based on non-specific adsorption and no marked difference in extraction amount was observed between MIP and NIP coating.

3.5. Coating extraction capacity

A series of metolachlor, propisochlor and butachlor mixed standard solutions of $0.005\text{--}5.0 \text{ mg L}^{-1}$ were used to investigate the extraction capacity of the MIP-coated stainless steel fiber. As shown in Fig. 4, MIP coating could extract three chloroacetanilide herbicides due to specific selectivities, and the extraction amount

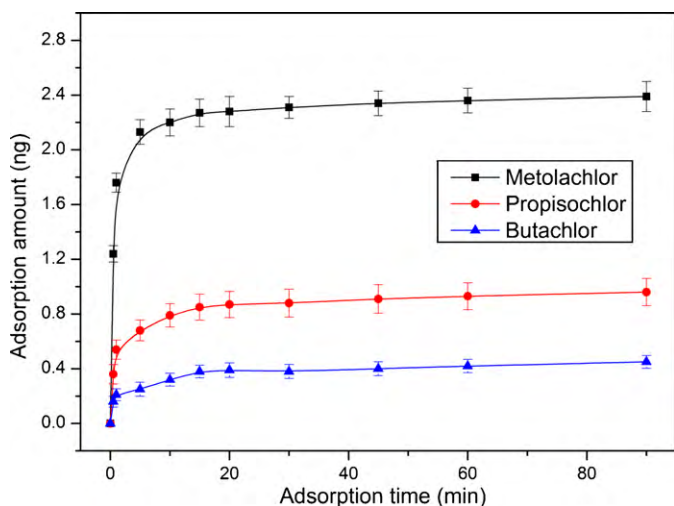


Fig. 5. Adsorption time curves of metolachlor MIP coating to metolachlor, propisochlor and butachlor mixed standard solution of $500 \mu\text{g L}^{-1}$.

of metolachlor was obviously larger than that of propisochlor or butachlor. When the concentration arrived at 5.0 mg L^{-1} , the extraction saturation approached and the extraction capacities of metolachlor, propisochlor and butachlor were about 4.6, 2.5 and 2.2 ng, respectively. To investigate the fiber-to-fiber extraction reproducibility, six MIP-coated stainless steel fibers were used to extract $500 \mu\text{g L}^{-1}$ mixed standard solution. RSDs of 5.7–8.5% for extraction amount were obtained satisfactorily.

3.6. Adsorption and desorption kinetics

Metolachlor, propisochlor and butachlor mixed standard solution of $500.0 \mu\text{g L}^{-1}$ were extracted with the MIP-coated fiber to perform the adsorption and desorption kinetics investigations, and the results were shown in Figs. 5 and 6, respectively. It was observed that the adsorption of three chloroacetanilide herbicides reached equilibrium quickly after 15 min which was remarkably shorter than the reported equilibrium time of 90 min for metolachlor and 300 min for butachlor with $100\text{-}\mu\text{m}$ PDMS commercial SPME fiber [40], or 30 min for prometryn, tetracycline and propranolol with 20- to $25\text{-}\mu\text{m}$ MIP coatings in our previous works [29–31]. This quick adsorption was mainly a result of the thin MIP coating with

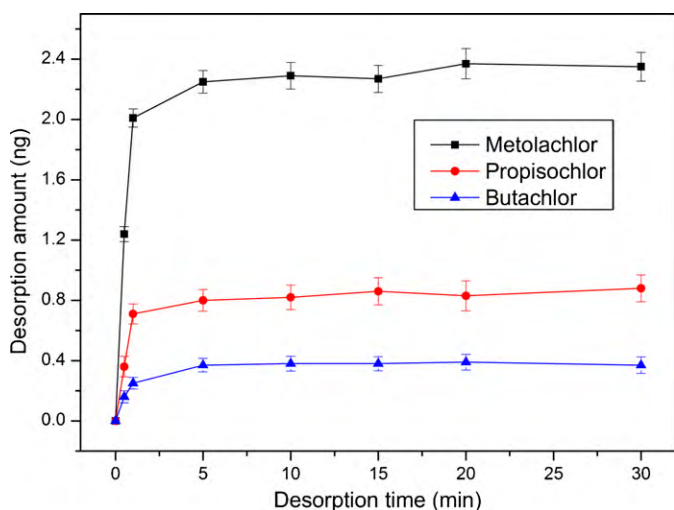


Fig. 6. Desorption time curves of metolachlor MIP coating to metolachlor, propisochlor and butachlor mixed standard solution of $500 \mu\text{g L}^{-1}$.

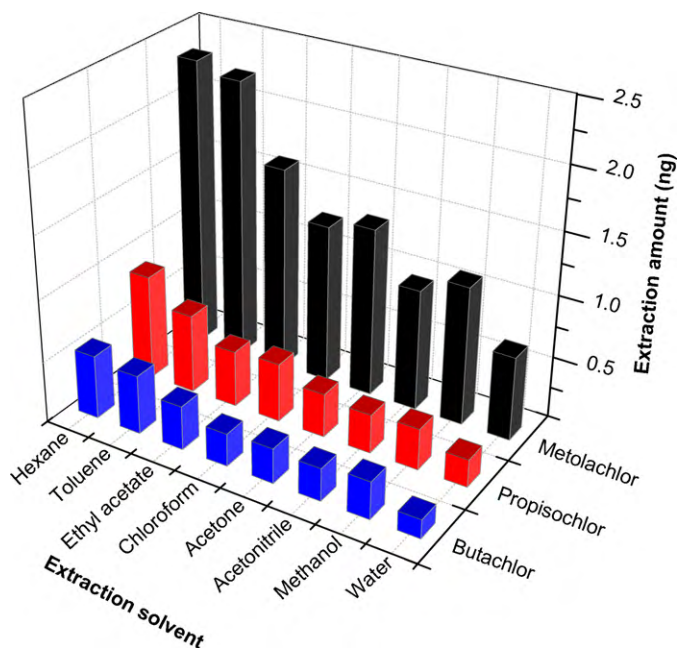


Fig. 7. Effect of extraction solvent to extraction amount of metolachlor, propisochlor and butachlor with MIP coating in mixed standard solution of $500 \mu\text{g L}^{-1}$.

thickness of about $15 \mu\text{m}$ which would guarantee the sufficient accessibility of selective recognition sites. Moreover, the highly cross-linked and porous morphological structure of MIP coating was another important contributing factor, by which the diffusion speed of analytes in coating could be enhanced. Similarly, thin thickness and favorable morphological structure would be beneficial to the quick desorption of analytes in the MIP coating. Consequently, about 87%, 81% and 66% of the adsorbed metolachlor, propisochlor and butachlor could be quickly desorbed within 1 min, respectively, and desorption reached equilibrium quickly after 5 min only.

3.7. SPME condition optimization

For the solvent optimization, hexane, toluene, ethyl acetate, chloroform, acetone, acetonitrile, methanol and water were studied with the MIP-coated stainless steel fiber for extraction of $500.0 \mu\text{g L}^{-1}$ metolachlor, propisochlor and butachlor mixed standard solution. Methanol was used as desorption solvent. As shown in Fig. 7, high extraction amounts of three chloroacetanilide herbicides were obtained with hexane and toluene, and in contrast, poor results were with polar solvents such as water and acetonitrile. According to previous work [31], the extraction would be disturbed in the polar solvent or aqueous solution due to their interferences to the hydrogen-bonding based recognition of MIP coating. Consequently, hexane was selected and it would be helpful for the exerting of MIP coating selectivity.

Desorption solvent and stirring speed were also investigated with $500.0 \mu\text{g L}^{-1}$ mixed standard solution. With methanol as the desorption solvent, three chloroacetanilide herbicides were desorbed with the highest amounts which were about 1.6–2.1 and 1.8–2.4 times as much as that with acetonitrile and mobile phase of acetonitrile–water (55/45, v/v), respectively. The optimization of stirring speed was performed at 0, 100, 250, 500, 750 and 1000 rpm, and the extraction amounts of three chloroacetanilide herbicides were all enhanced with the increase of stirring speed and arrived at equilibrium after 750 rpm.

Table 2
Recoveries of metolachlor, propisochlor, and butachlor in spiked soybean and corn samples.

Compound	Soybean						Corn					
	20.0 $\mu\text{g L}^{-1}$		100.0 $\mu\text{g L}^{-1}$		500.0 $\mu\text{g L}^{-1}$		20.0 $\mu\text{g L}^{-1}$		100.0 $\mu\text{g L}^{-1}$		500.0 $\mu\text{g L}^{-1}$	
	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
Metolachlor	85.3	6.8	92.8	5.8	93.4	5.6	88.6	5.9	90.7	4.1	96.4	4.3
Propisochlor	78.9	7.2	85.6	7.4	89.8	5.7	80.1	6.5	88.5	6.3	90.6	5.3
Butachlor	/	/	74.3	7.6	87.6	6.9	/	/	70.7	6.2	84.5	5.8

"/" Butachlor could not be detected in extracts of 20.0 $\mu\text{g L}^{-1}$ spiked soybean and corn samples with MIP coating.

3.8. Linearity, limit of detection and precision

The linearity of MIP-coated SPME–HPLC method was studied with a series of metolachlor, propisochlor and butachlor mixed standard solutions under above optimized conditions. The linear ranges of 10–1000, 50–1000 and 100–1000 $\mu\text{g L}^{-1}$ with correlation coefficient of 0.9906, 0.9892 and 0.9875 were obtained for metolachlor, propisochlor and butachlor, respectively. The limits of detection (LODs) were calculated at a signal-to-noise ratio of 3, 3.0, 9.6 and 38 $\mu\text{g L}^{-1}$ were achieved for metolachlor, propisochlor and butachlor, respectively, which could meet metolachlor maximum residue limit of 70 $\mu\text{g L}^{-1}$ recommended by the United States environmental protection agency (USEPA) lifetime health advisory

[43]. The method precision was monitored with 100.0 $\mu\text{g L}^{-1}$ mixed standard solution and the RSDs of extraction amounts of metolachlor, propisochlor and butachlor were 3.2, 4.9 and 9.5% ($n=6$), respectively.

3.9. Real sample analysis

To validate the practical performance of MIP-coated stainless steel fiber in real samples with complex matrix, soybean and corn were selected for the spiking analysis at three levels of 20, 100, 500 $\mu\text{g L}^{-1}$ with metolachlor, propisochlor and butachlor as analytes. The chromatograms of 20 $\mu\text{g L}^{-1}$ spiked soybean and corn sample solutions and their extracts with MIP and commercial PDMS/DVB, PDMS and PA coatings were shown in Fig. 8. It was indicated that direct HPLC analysis with traditional UV detector could not monitor metolachlor, propisochlor and butachlor of 20 $\mu\text{g L}^{-1}$ or lower concentrations. The adoption of SPME with commercial PDMS/DVB coating for sample pretreatment was advantageous to metolachlor detection, while PDMS and PA coatings did not show any improvement. In contrast, metolachlor and propisochlor monitoring could be improved markedly with MIP coating, and the interferences from sample matrix were reduced simultaneously. For Butachlor, it could be detected in 100 and 500 $\mu\text{g L}^{-1}$ spiked sample solutions when extracted with MIP coating. According to peak area correction with 1.0 mg L^{-1} standard solution, the enrichment factors of metolachlor, propisochlor and butachlor at 100 $\mu\text{g L}^{-1}$ level with MIP coating were 54–60, 27–31 and 15–20, whereas 7.8–11, 3.9–4.5 and 2.7–3.1 were obtained with PDMS/DVB coating, respectively. The recoveries of metolachlor, propisochlor and butachlor in spiked soybean and corn samples were 85.3–96.4, 78.9–90.6 and 74.3–87.6%, respectively, as shown in Table 2. It was indicated that the MIP-coated stainless steel fiber could be used for selective separation and enrichment of trace chloroacetanilide herbicides in complicated samples.

4. Conclusions

In this paper, MIP coating was firstly coated on the surface of stainless steel fiber through chemical bonding. The problem lifetime of SPME fibers restricted by the silica fiber fragility was solved with stainless steel fiber. By means of pretreatment investigation for stainless steel fiber and two-stage optimization strategies for polymerization conditions, homogeneous and porous MIP coating was obtained with good thermal and chemical stabilities. Specific selectivities to chloroacetanilide herbicides and metolachlor metabolites were found with the MIP-coated SPME stainless steel fiber, and good extraction capacities for metolachlor, propisochlor and butachlor could be achieved. For spiked soybean and corn sample analysis, the sensitivities of metolachlor, propisochlor and butachlor were improved significantly, and the interferences from plant matrix were eliminated obviously. The spiking recoveries of metolachlor, propisochlor and butachlor were 85.3–96.4, 78.9–90.6 and 74.3–87.6%, respectively.

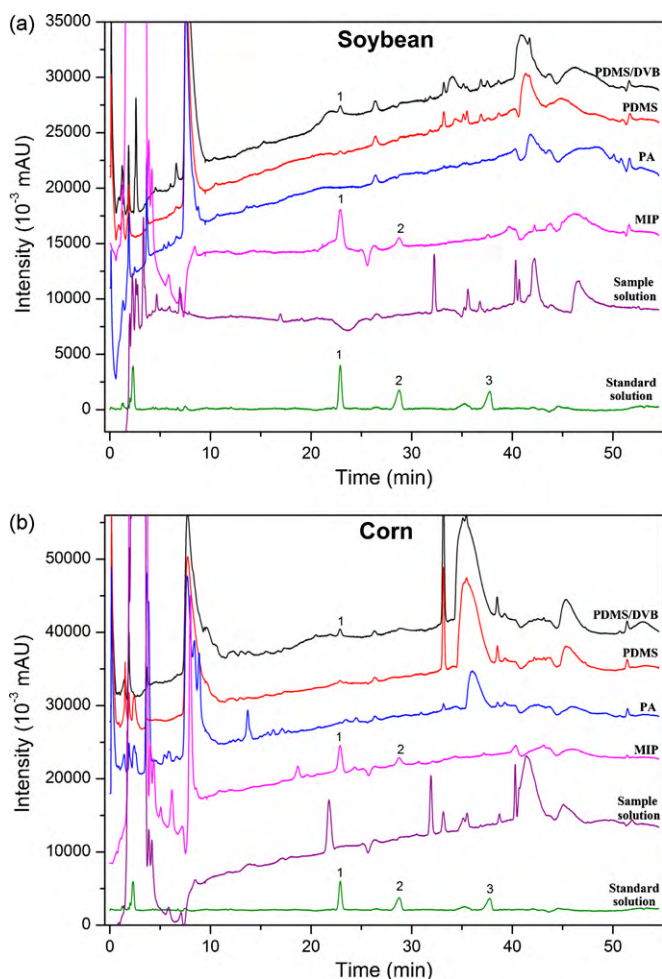


Fig. 8. Chromatograms of 1.0 mg L^{-1} metolachlor, propisochlor and butachlor mixed standard solution, 20.0 $\mu\text{g L}^{-1}$ spiked soybean and corn sample solutions after ultrasonic extraction and its extracts with MIP or commercial PDMS/DVB, PDMS and PA coatings. (1) metolachlor, (2) propisochlor, (3) butachlor, injection volume for standard solution and spiked sample solutions: 10 μL .

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at [doi:10.1016/j.chroma.2010.07.011](https://doi.org/10.1016/j.chroma.2010.07.011).

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