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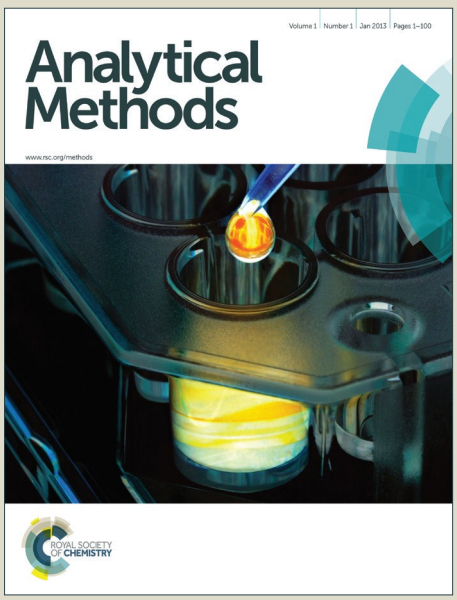

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Analysis of sulfonylurea herbicides in grain samples by molecularly imprinted polymers on the surface of magnetic carbon nanotubes extraction coupled with HPLC

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A new strategy for the isolation and enrichment of the sulfonylurea herbicides in grain samples was obtained by magnetic molecularly imprinted polymers (MMIPs) using carbon nanotubes as matrix, nicosulfuron as template molecule, 3-aminopropyltriethoxysilane as functional monomer, tetraethyl orthosilicate as cross-linker and cetyl trimethylammonium bromide as dispersant. The characteristics of the MMIPs were assessed by transmission electron microscopy, vibrating sample magnetometry, X-ray diffractometer, elemental analyzer, Brunauer-Emmett-Teller method and Fourier transform infrared spectroscopy. The binding characteristics of imprinted materials were researched including isothermal adsorption experiment, kinetics adsorption experiment and the selectivity experiment. The recoveries of four sulfonylurea herbicides were more than 80.0% and the detection limits were 0.0107, 0.0151, 0.0147 and 0.0123 μg g^{-1}, respectively. RSDs of intra-day and inter-day precisions in the range of 0.7–2.4% and 5.3–8.4% were obtained, respectively. The results demonstrate that MMIPs are promising for the preconcentration, purification and analysis of sulfonylurea herbicides in grain samples.

Keywords: Carbon nanotubes, Magnetic molecularly imprinted polymers, High performance liquid chromatography, Sulfonylurea herbicides, Grains

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1. Introduction

Sulfonylurea herbicides (SUHs) are a new generation of environmentally compatible herbicides introduced by DuPont Crop Protection in 1982 and considered as one of the most useful classes of herbicides.[1] Because of their high herbicidal activity and low mammalian toxicity, they are extensively used for weed control in many agricultural crops such as wheat, flax, corn, potato and turnip.[2] However, these economic benefits are not without risk to human health and environmental damage. Nicosulfuron, metsulfuron-methyl, chlorsulfuron and halosulfuron-methyl are always used in grains and frequently researched in previous studies.[3, 4] Due to their high solubility in water, high mobility and slow degradation, they are detected widely in plants. Concerns have been raised by the public and regulatory authorities.[5, 6] The European Union Commission has defined a minimum residue limits (MRLs) for SUHs in grain samples at a level of 0.05 mg kg^{-1}.[7] Therefore, efficient, reliable and sensitive analytical methods are indispensable for the detection of SUHs residues.

Now several analytical methods have been described to detect SUHs.[8-15] In these methods, high-performance liquid chromatography (HPLC) is the commonly used technique for the separation and quantification of SUHs residues in different matrices. Due to the low presence and complexity in sample constituents, a reliable sample pretreatment procedure for the clean-up and enrichment of analytes before HPLC analysis is necessary and crucial step for the determination of SUHs in complex samples, such as soil, grains and milk. Pretreatment procedures related to SUHs generally include micro-porous membrane liquid–liquid extraction (MMLLE),[8] continuous-flow liquid membrane extraction (CFLME),[9] solid-phase extraction (SPE),[10-12] matrix solid phase dispersion (MSPD),[13] ultrasound-assisted surfactant-enhanced emulsification microextraction (UASEME)[14] and QuEChERS.[15] Some of the pretreatment procedures were limited with the disadvantages such as time consuming, labor-intensive, poor immunity from interference and the use of a large amount of organic solvents. Besides, other chemicals extracted companying with the target analytes can interfere with the detection of the targets.

In recent years, considerable attentions have been paid to molecular imprinting technique as an

excellent and simple approach to producing artificial receptors with predetermined ligand selectively. The technique is based on the copolymerization of a monomer with cross-linker in the presence of a template molecule, so the positions and orientations of the functional residues of the monomer are immobilized in the polymers, which are complementary to the template molecule in size, shape and interaction patterns. Thus, molecularly imprinted polymers (MIPs) can rebind template molecule with very high affinity and specificity. Until now, MIPs have been widely used in many areas such as solid phase extraction,[16] various sensor strategies,[17] chromatographic separation,[18] catalysis studies,[19] pharmaceutical analysis,[20] etc. This technique is a conceptually simple and straightforward method of applying to a wide variety of target molecules.[21, 22] Moreover, the MIPs also have been prepared to adsorb SUHs.[23-28] However, most of the MIPs were prepared by bulk polymerization in the study,[23-26] which exhibited some limitations including incomplete template removal, slow mass transfer, small binding capacity and irregular polymers shape.

Compared with traditional MIPs, magnetic MIPs (MMIPs) nanoparticles have been considered as ideal adsorbent materials and received increasing attention, because the MMIPs have unique magnetic property which enables them to be easily separated from the matrix under an external magnetic field after adsorption and recognition. Meanwhile, MMIPs can provide the selectivity for the target molecules. Recently, MMIPs have been prepared and used to detect 2,4-dichlorophenoxyacetic acid,[4] tetracycline[29] and enrofloxacin.[30] The results show that MMIPs have high selectivity and strong anti-interference ability.

Carbon nanotubes (CNTs) are always chosen to prepare MMIPs as matrix materials due to their extraordinarily high aspect ratios, unique atomic structures and probable functional groups after functionalization.[31] CNTs decorated with magnetic nanoparticles on their external surface will combine the high adsorption capacity of CNTs with the convenient separation of magnetic materials. Due to the large surface areas and the binding sites in the outer layer of the MMIPs, the accessibility of template molecule would be improved and the binding time would be reduced.[32] Moreover, MMIPs could selectively recognize the template molecule in complex matrixes.[33]

The prepared MMIPs were used as adsorbents for separation of SUHs including nicosulfuron, metsulfuron-methyl, chlorsulfuron and halosulfuron-methyl which were often used in grain samples.[34] The characteristics of the MMIPs, binding properties and extraction conditions were investigated in this paper.

2. Materials and methods

2.1. Chemicals and reagents

The standards of nicosulfuron, metsulfuron-methyl, chlorsulfuron and halosulfuron-methyl (Fig. S1), tetraethoxysilicane (TEOS) and 3-aminopropyltriethoxysilane (APTES, kh-550) were purchased from Aladdin (Shanghai, China). The carbon nanotubes (CNTs) were purchased from Nanoport (Shenzhen, China). Ammonia hydroxide (25%) and nitric acid (65%) were purchased from Guangfu (Tianjin, China). Cetyltrimethylammonium bromide (CTAB), acetonitrile, ethanol, methanol, ethylene glycol (EG), acetic acid (HAc), ferric chloride crystal ($FeCl_3 \cdot 6H_2O$) and sodium acetate anhydrous (NaAc) were purchased from Kermel (Tianjin, China). All reagents used were of analytical grade. Acetonitrile of chromatographic grade was obtained from Fisher (Pittsburgh, PA, USA). High purity water was obtained from a Milli-Q Water System (Millipore, MA, USA).

2.2. Instruments

The surface groups on the as-synthesized nanoparticles were measured with a 360 Fourier transform infrared (FT-IR) spectrometer (Nicolet, Madison, WI, USA). The morphology characteristic of MMIPs was measured with a Hitachi H-7650 transmission electron microscope (TEM) (Matsudo, Japan). The superparamagetism of the material was measured with a vibrating sample magnetometer (VSM) (Quantum Design Instrument, San Diego, CA, USA). The structure of the powder samples were characterized by a DX-2600 X-ray diffractometer (XRD) (Dandong, China). The element contents of MMIPs were measured by elemental analyzer (vario EL, Elementar Analysensysteme GmbH, Germany). A Brunauer-Emmett-Teller (BET) surface area instrument (Quadrasorb SI-MP, Quantachrome, Florida, USA) was also used to measure the

porosity of the materials. A KQ5200E ultrasonic apparatus (Kunshan Instrument, Kunshan, China), DZKW-C thermostatic bath (Shanghai, China), DF-101S thermostatic oil bath (Yarong Instrument, Zhengzhou, henan, China), TG 16-WS centrifuge (Xiangyi, Changsha, China) and SHA-B shaking table (Shengtang, Jintan, China) were used. Chromatographic analysis was performed on a LC-15C high performance liquid chromatograph with a UV detector (Shimadzu, Kyoto, Japan). A Zorbax SB-C18 column (150 mm × 4.6 mm I.D., 5 μm) was used as an analytical column (Palo Alto, CA, USA).

2.3. Preparation of standard solutions

Individual analytical standard solutions of herbicides were prepared by exactly weighing and dissolving four kinds of SUHs in acetonitrile with the concentration of 1.0 mg mL^{-1}. Furthermore, the standard solutions were protected against light and stored at 4 ^{o}C in refrigerator. Work standard solutions were daily prepared by the serial dilution of the stock solutions.

2.4. Grain samples

In order to identify the good application and anti-interference of the polymers, grains with different contents of pigment and protein were selected. The grains such as soybean, corn and wheat were randomly purchased from the local markets (Harbin, China) and stored at 4 ^{o}C in refrigerator. The samples were ground into power before they were used. One soybean sample was checked to be free of SUHs according to the method reported in National Standard of China.[35] And it was used as blank sample for calibration and validation purposes. The spiked grain samples were prepared by adding SUHs standard solutions into grain samples.

2.5. Synthesis of MMIPs

2.5.1. Synthesis of MCNTs

The impurities such as amorphous carbon and metallic catalyst in the CNTs (0.5 g) were removed using HNO_3 contained in a three neck flask with vigorous stirring (500 rpm) at 90 ^{o}C for 4 h. Then the suspension was filtered through a filter to recover the CNTs, followed by washing repeatedly with high purity water until the pH reached 7.0, and then dried under vacuum at 70 ^{o}C overnight for

further use.

The preparation of MCNTs was carried out according to previous study.[34] Activated CNTs (0.4 g), $FeCl_3 \cdot 6H_2O$ (2.4 g) and NaAc (3.4 g) were added into ethylene glycol (70 mL) under ultrasonication for 10 min. The homogenous black solution obtained was transferred to a Teflon-lined stainless-steel autoclave and sealed to heat at 200 ^oC. After reaction for 8 h, the autoclave was cooled down to room temperature. The MCNTs obtained were separated from the solvent by an external magnetic field and washed several times with ethanol and water. Then MCNTs were dried in vacuum at 70 ^oC for 24 h.

2.5.2. Coating SiO_2 on MCNTs (MCNTs@SiO_2)

The SiO_2 coating on the CNTs was performed as followed. MCNTs (250 mg), APTES (0.5 mL), CTAB (100 mg) and water (50 mL) were added in a conical flask and sonicated for 20 min, and then stirred for another 3 h. TEOS (4.0 mL), water (3.0 mL) and ethanol (50 mL) were added in the other conical flask and treated exactly as the mixture in the first conical flask. The mixture in two conical flasks was mixed together and then sonicated for 30 min, followed by stirring for 10 min. Ammonia hydroxide was added dropwise until the pH value reached 9.5. After the SiO_2 was coated on the MCNTs, the product was separated from the solvent by an external magnetic field and washed with water and ethanol to remove the surfactants thoroughly. Finally, the product was dried in vacuum at 60 ^oC overnight.

2.5.3. Preparation of MMIPs

To prepare the MMIPs, nicosulfuron (0.4 mmol) was dissolved in ethanol (60 mL) and mixed with APTES (4 mL). The mixture was stirred for 20 min, and then TEOS (4 mL) was added. After stirred for 5 min, MCNTs@SiO_2 (1.2 g) and 1.0 mol L^{-1} HAc (1.0 mL) as catalyst were added. The mixture was incubated for 10 h at room temperature. The product was separated from the solvent by an external magnetic field and washed with water and then dried in a vacuum oven at 60 ^oC for overnight. Thus, the complex of nicosulfuron and APTES was grafted on the surface of the MCNTs@SiO_2. After the polymerization, the product was separated with a permanent magnet and

then washed with methanol/acetic acid (8:2, v/v) by Soxhlet extraction until the nicosulfuron could not be detected by HPLC. Finally, the polymers were washed with water several times and dried at 60 ^{o}C.

For comparison, the magnetic non-imprinted polymers (MNIPs) were also prepared following the same procedure of the synthesis of MMIPs without nicosulfuron.

2.6. Binding experiments

Template molecule nicosulfuron was used as a representative to investigate the recognition property of MMIPs. The isothermal adsorption experiment was carried out by adding 20 mg MMIPs or MNIPs and 2 mL nicosulfuron standard solution with various concentrations from 0.1 to 500 μg mL^{-1} into a tube and shaking for some time. The adsorbent was separated by a permanent magnet and residual nicosulfuron in supernatant was detected by HPLC. The amount of nicosulfuron bound to the MMIPs or MNIPs was calculated by subtracting the free mass from initial mass of nicosulfuron.

In kinetic adsorption experiments, MMIPs (20 mg) or MNIPs (20 mg) were mixed with 2 mL of nicosulfuron standard solution at a concentration of 200 μg mL^{-1} and incubated at regular time intervals, and then the supernatants and polymers were separated by permanent magnet. The nicosulfuron concentrations of the supernatants were measured by HPLC.

The selectivity experiment was carried out by using metsulfuron-methyl, chlorsulfuron and halosulfuron-methyl as the structural analogs of nicosulfuron, cyanazine was used as reference compound due to its usual application in grains during the binding process. The MMIPs or MNIPs (20 mg) were placed in 2 mL of 500 μg mL^{-1} of SUHs and cyanazine standard solution, respectively. The mixture was stirred at room temperature for 24 h. Then the supernatants and polymers were separated by permanent magnet. The supernatant was detected by HPLC.

2.7. Separation of nicosulfuron in grain samples

Acetonitrile (6 mL) was added to grain samples (1.0 g), then the mixture was shaken for 30 min at room temperature, the supernatant solution was separated by centrifugation. MMIPs (100 mg)

were added to the supernatant solution, and then shaken for 20 min at room temperature. Next, the supernatants and polymers were separated by permanent magnet. Finally, MMIPs which adsorbed target molecule were eluted with a mixture of methanol/acetic acid (95:5, v/v). The eluent was evaporated to dryness under nitrogen gas at 40 ^{o}C, and then the residue was reconstituted with 1.0 mL of liquid chromatography mobile phase for HPLC analysis.

2.8. Chromatographic analysis

The SUHs were analyzed by HPLC with UV detector and C18 column. The mobile phase was 45% acetonitrile aqueous solution with 0.6% acetic acid delivered at a flow rate of 1.0 mL min^{-1}. The injection volume was 20 μL, the column temperature was room temperature and the column effluent was monitored at 254 nm.

3. Results and discussion

3.1. Preparation of MMIPs

The synthesis of MMIPs via a multistep procedure is involved in the formation of template (nicosulfuron)-aminosilica monomer (APTES) complex, silica–shell deposition on the surface of MCNTs, MIP-functionalized onto the silica surface and extraction of nicosulfuron to generate the recognition sites (Fig. 1). This method contained two sol–gel processes: one was to transform silica shell to the surface of MCNTs using TEOS in the presence of cationic surfactant CTAB to produce the MCNTs@SiO_2 with core–shell structure; the other was to anchor an MIPs shell on the surface of MCNTs@SiO_2 using HAc as the catalyst, nicosulfuron as template molecule, APTES as functional monomer and TEOS as cross-linker.[36]

As a material of high degree of crosslinking, MIPs were synthesized in the presence of nicosulfuron, TEOS and APTES. After nicosulfuron was removed, the specific recognition sites for the template are formed. These sites are complementary to the template molecule in the size, shape and functional group, which makes sure the specific hole have high adsorption and selective to target molecule. The interaction through hydrogen bond between the -NH_2 of APTES and -C=O and

-NH of nicosulfuron is the base of the formation of the specific adsorption sites on MIPs. The binding mechanism is given in Fig. 1 which in detail describes how functional monomer and template react through hydrogen bond.

3.2. Characterization studies

The TEM images of the CNTs and MMIPs are compared in Fig. 2a and b. The TEM images show that after a series of reactions the thick of CNTs increased.

The porosity of the prepared materials was investigated by a nitrogen adsorption-desorption experiment. The specific surface areas of the MMIPs with and without CNTs, MNIPs, CNTs and MCNTs were 178.24, 93.94, 160.37, 138.76 and 122.85 m^2 g^{-1}, respectively. The average pore diameters of the MMIPs with and without CNTs, MNIPs, CNTs and MCNTs were 3.2, 1.5, 2.8, 2.1 and 1.7 nm, respectively. Generally, the bigger specific surface area and average pore size are, the stronger adsorption capacity is. The results suggested that the adsorption capacity of MMIPs with CNTs to SUHs is better than the other materials due to the existence of molecularly imprinted sites and CNTs.

The FT-IR spectra of CNTs, MMIPs and MNIPs are shown in Fig. 2c. The band at 1635 cm^{-1} is attributed to the water occluded in the solid. This is commonly obtained in silica gel obtained sol-gel process. The strong peak at about 1088 cm^{-1} is attributed to the stretch of Si–O–Si, the peak at 468 cm^{-1} is also due to Si–O–Si and the –OH vibration is detected at 3446 cm^{-1}. The above peaks indicated the formation of silica film on the surface of CNTs. The adsorption band around 2974 cm^{-1} unveiled the stretching vibration of C–H group. The characteristic peak at 554 cm^{-1} is assigned to the Fe–O bond. There are only two peaks at 1635 and 3446 cm^{-1} in the FT-IR spectrum of CNTs. And the FT-IR spectra of MMIPs and MNIPs almost have no differences.

An elemental analysis of the MMIPs has been performed. The quality percentage of nitrogen is 6.37%, which evidences the presence of the amino group in the MMIPs.

The hysteresis loop of MMIPs is shown in Fig. 2d, which indicates the magnetic saturation value is about 23.68 emu g^{-1} and the MMIPs are superparamagnetic. Magnetization of the MMIPs

achieved rapid and convenient separation from aqueous solutions under a magnetic field. The inset in Fig. 2d shows MMIPs suspension in solution becoming clear after applying an external magnet and this response vanished upon the removal of the magnetic field.

Fig. 2e illustrates the X-ray power diffraction (XRD) patterns of Fe_3O_4 and MMIPs, which displays several relatively strong reflection peaks in the 2θ region between 10^o~80^o. As shown in Fig. 2e, the discernible six characteristic peaks for Fe_3O_4 (2θ=30.2^o, 35.6^o, 43.3^o, 53.5^o, 57.2^o and 62.8^o) are observed for the sample, and the peak positions can be indexed to (220), (311), (400), (422), (511), and (440), respectively. The peak at 2θ = 25.8^o is the typical peak of carbon nanotubes. The results indicate that the crystal structure of the magnetite were unchanged and essentially maintained during the polymerization process.

After series of characterizations, the results prove that the Fe_3O_4 nanoparticles were deposited onto the surface of the CNTs and the MIPs were formed on the surface of Fe_3O_4 and CNTs.

3.3. Binding characteristics of imprinted materials

3.3.1. Isothermal adsorption experiment

As shown in Fig. S2a, the binding isotherm results of MMIPs and MNIPs rebinding performance are plotted. Nicosulfuron adsorbed by the polymers increased with increase of the initial nicosulfuron concentration. The maximum binding amount of MMIPs toward nicosulfuron was much higher than that of MNIPs, indicating the significant preferential adsorption of template molecule for MMIPs. In order to further study the specificity and evaluate the adsorption parameters of MMIPs and MNIPs, Scatchard analysis was used to discuss the binding characteristics.[37] Scatchard equation is expressed as follows:

$$\frac{Q}{C} = \frac{Q_{max} - Q}{K} \quad (1)$$

Where Q is the amount of nicosulfuron bound to MMIPs at equilibrium, Q_{max} is the apparent maximum adsorption capacity, C is the free analytical concentration at equilibrium and K is the dissociation constant. The values of K and Q_{max} could be calculated from the slope and intercept of the linear curve plotted as Q/C versus Q.

The Scatchard analysis of MMIPs was performed. It was observed that two straight lines were obtained in the plot region (Fig. S2b), which indicated that there existed two kinds of binding sites of high and low affinity. The linear regression equations for the left and right slope of the curve are Q/C = 5.6660 - 1.0681Q (R^2 = 0.9985) and Q/C = 0.3757 - 0.01171Q (R^2 = 0.9762). From the slope and the intercept of the biphasic curve obtained, the values of K were 0.002291 and 0.2083 mmol L^{-1}, Q_{max} were 5.30 and 32.11 mg g^{-1}, respectively.

The Scatchard analysis of MNIPs is showed in Fig. S2c, which indicated that there existed one kind of binding site. The linear regression equation is Q/C=0.2390 - 0.02362Q (R^2=0.9702), the value of K was 0.1032 mmol L^{-1} and the Q_{max} was 10.12 mg g^{-1}.

The adsorption of CNTs, MCNTs, MMIPs with and without CNTs were investigated. The results showed that nicosulfuron binding amounts of CNTs (7.22 mg g^{-1}), MCNTs (4.37 mg g^{-1}) and MMIPs without CNTs (4.83 + 17.37 mg g^{-1}) were obviously lower than that of MMIPs (5.30 + 32.11 mg g^{-1}).

3.3.2. Kinetics adsorption experiment

As shown in Fig. S3a, the adsorption amount of nicosulfuron onto MMIPs increased with increase of the adsorption time, especially increased rapidly in the first 20 min. After that the rebinding rate started to decrease and finally almost had no changes in the subsequent rebinding process. The adsorptive phenomenon by MNIPs was similar with MMIPs. However, the amount of nicosulfuron adsorbed by MNIPs was obviously lower than that by MMIPs, which was attributed to the contribution of the selective cavities of the MMIPs.

To investigate the controlling mechanisms of binding, the first-order and second-order kinetics model were studied. They can be expressed as following equations, respectively:[37]

$$\log\left(Q_{eq} - Q_t\right) = \log Q_{e1} - \frac{k_1 t}{2.303} \quad (2)$$

$$\frac{t}{Q_t} = \frac{1}{k_2 Q_{e2}^2} + \frac{t}{Q_{e2}} \quad (3)$$

In Eqs. (2) and (3), Q_{eq} (mg g^{-1}) and Q_t (mg g^{-1}) are the amount of nicosulfuron adsorbed at

equilibrium and in time t (min), respectively. Q_{e1} (mg g^{-1}) and Q_{e2} (mg g^{-1}) are the theoretic adsorption capacity of the first-order and second-order kinetics model. k_1 (min^{-1}) and k_2 (g mg^{-1} min^{-1}) are the equilibrium rate constant of first-order and second-order sorption, the values of them can be calculated from the plot of $\log(Q_{eq} - Q_t)$ versus t and t/Q_t versus t, respectively. The results of kinetic analysis were shown in Fig. S (3b and c) and Table 1. According to the higher values of R^2 (0.9999 and 1.0000) and closer values between Q_e and Q_{eq}, second-order kinetics model was considered as the better-fit model.

3.3.3. The selectivity of MMIPs

Selective recognition toward the template molecule, which depends on the imprinted cavities in complement to the size, shape, and functionality of the template molecule, is an important property for a novel imprinted material. The results of the selectivity were showed in Table 2. Compared with MNIPs, the MMIPs exhibited higher rebinding selectivity for nicosulfuron and its structural analogs. However, the amounts of cyanazine adsorbed by MMIPs and MNIPs were similar. To further investigate the selective recognition of the MMIPs, the selectivity parameters were measured. The interrelated adsorbed coefficients were calculated by the following equations:

$$K_d = \frac{Q}{C} \tag{4}$$

In Eq. (4), K_d (L g^{-1}) represents the distribution coefficient; Q (mg g^{-1}) and C (mg L^{-1}) represent the adsorption capacity and initial concentration of the solution, respectively. The selectivity coefficient K' and relative selectivity coefficient K'' values could be calculated by the following equations:

$$K' = \frac{K_{d(S)}}{K_{d(C)}} \tag{5}$$

$$K'' = \frac{K'_M}{K'_N} \tag{6}$$

Where $K_{d(S)}$ and $K_{d(C)}$ represent the static distribution coefficients of SUHs and cyanazine, respectively; K'_M and K'_N are selectivity coefficients of MMIPs and MNIPs, respectively.

The values of K_d, K' and K'' were shown in Table 3. The relative selectivity coefficients of SUHs toward cyanazine were in the range of 1.78–2.00. This might be resulted from the imprinting effect, molecular size recognition and the interactions between functional groups of the targets and imprinted cavities.

3.4. Optimization of the conditions

In order to evaluate the applicability of MMIPs for extraction and determination of SUHs in grain samples, the parameters affecting the performance of the extraction, such as the amount of MMIPs, adsorption time, shaking rate, desorption solvent and elution times (Fig. 3) were investigated. Thus, it is necessary to optimize conditions. The extraction conditions were optimized by analyzing the spiked soybean samples. All experiments were performed using 6 mL extracting solution to extract 1.0 g soybean spiked with 2.0 mg kg^{-1} SUHs. When one parameter was changed, the other parameters were fixed at their optimized values. In this work, acetonitrile was used to extract four kinds of SUHs from soybean samples according to the National standard method used in China.[35]

3.4.1. The amount of MMIPs

Different amounts of MMIPs ranging from 10 to 100 mg were applied to extract the SUHs from soybean samples (Fig. 3a). The results indicated that the recoveries of SUHs increased with the increasing of the amounts of MMIPs at first, when the amount was above 90 mg, further increasing the amounts of MMIPs gave no improvement for recoveries of SUHs.[33]

Furthermore, the adsorption and desorption cycles were repeated at least 10 times by the using the same MMIPs to evaluate the reusability of multifunctional nanoparticles. When the reuse of MMIPs was more than 10 times, the capacity of the MMIPs adsorption began to decrease. This was mainly resulted from the partial destruction of the imprinted cavity, and indicated MMIPs owned stable recyclable activity. Meanwhile, the batch-to-batch variation of the newly prepared MMIPs has been researched. The experiments prove that there are no obvious differences of these materials for adsorbing the SUHs. The satisfactory recoveries can be obtained.

3.4.2. Adsorption time

The experimental results indicated that the adsorption time had an obvious effect on the target analytes adsorption. The adsorption process of SUHs must be equilibrated for enough time to obtain satisfactory recoveries. The effect of the extraction time from 1 to 80 min on the recoveries of SUHs was investigated (Fig. 3b). The recoveries of SUHs increased with increasing contact time up to 20 min. Further increase in contact time does not result in significant increment in recoveries but leads to a plateau. Therefore, in this study, the extraction time of 20 min was chosen to obtain the complete extraction.[33]

3.4.3. Shaking rate

The experimental results indicated that the shaking rate obviously affect the adsorption efficiency of the MMIPs. The effect of the shaking rate from 0 to 240 rpm on the recoveries of SUHs was investigated (Fig. 3c). The recoveries of SUHs increased with increasing shaking rate to 240 rpm. Therefore, in this study, the shaking rate of 240 rpm was chosen for obtaining the complete extraction.

3.4.4. Desorption solvent

It is important to decrease the matrix interference after the extraction and improve the selective binding of the SUHs. A series of desorption solutions,[20] ethanol, methanol and acidified methanol were used to make the desorption condition optimum and obtain the highest recoveries of SUHs (Fig. 3d). Poor recoveries were found by using ethanol. The best recoveries were obtained using 1.0 mL mixture of methanol/acetic acid (95:5, v/v) as desorption solvent.

3.4.5. Elution times

Elution times were investigated to obtain the best elution efficiency. One milliliter elution solvent was used every time for eluting SUHs from MMIPs. The results shown in Fig. 3e indicated that SUHs were almost eluted from the MMIPs when the polymers were eluted three times.

3.5. Method validation

The method validation was done according to the European Commission Decision.[38]

The specificity of the method was checked by analyzing different blank grain samples. No

interfering peaks and false positive results were observed in the blank chromatograms, which indicated that the selectivity of the method is good.

The stability of analytes in standard solution and extract was also checked. Stock solutions of the analyte standards (1.0 mg mL^{-1}) were stored in refrigerator at 4 ^{o}C and found to be stable for two months. Work standard solutions were daily prepared by diluting the stock solutions with water. The analytes in the extract were found to be stable at room temperature for 24 h.

The SUHs were separated and detected by HPLC. The chromatograms of SUHs standard solution, blank soybean sample and spiked soybean sample were shown in Fig. 4. The sensitivity of the method was described by the limit of detection (LOD). The LOD was defined as three times ratio of signal to noise.[29] The LODs of four SUHs were in the range of 0.0107–0.0151 mg kg^{-1} (Table 3). The maximum residue limits (MRLs) of SUHs were 0.05 mg kg^{-1} in grain samples established by the European Union.[7] Compared with that, the values of LOD proved that the method was sensitive. It is conceivable that the sensitivity may be improved by using HPLC-MS.

To investigate the precision, relative standard deviations (RSDs) of intra-day and inter-day precisions at three different fortified concentrations of 0.05, 0.5 and 5.0 mg kg^{-1} were measured. The intra-day precision was evaluated with six replicates of quality control samples analyzed in the same day, and the inter-day precision was evaluated by analyzing this sample once a day during six consecutive days. RSDs of intra-day and inter-day precisions in the range of 0.7–2.4% and 5.3–8.4% were obtained, respectively. In all three fortified levels, recoveries of SUHs ranged from 82.2% to 98.0%.

Different analytical methods for determination of SUHs were summarized briefly in Table 4. As can be seen, the recoveries, LODs and RSDs of this method were comparable to other methods.[3, 13, 32, 39, 40, 41] This material had selectivity for target analytes because the molecularly imprinting technique was used. Moreover, traditional column passing process was avoided due to magnetism of adsorbent and the separation time was saved. Among other methods, matrix solid phase extraction (MSPD) method seemed to be easier and faster, nevertheless the process needed manual

grind, making the method strenuous. The comparison proved that the method in this study was convenient, rapid and efficient for analyzing SUHs in grains.

3.6. Application to real samples

In order to evaluate the applicability and reliability of the proposed method, it was applied to determine SUHs in different grains including two soybean samples, two corn samples and two wheat samples. In one corn sample, the chlorsulfuron was found at the level of 0.086 mg kg^{-1}, while SUHs residues were not found in other samples. The recoveries of SUHs were studied in the real samples by analyzing the spiked samples under the optimized conditions. The concentration added into the samples was 1.0 mg kg^{-1}. The following steps were according to the procedure described in experiment. The matrix-matched calibration curves which were obtained by spiking SUHs into different blank grains (soybean, corn and wheat) extract were used to calculating the SUHs recoveries in the corresponding grain samples. The results showed that MMIPs had good recoveries (80.1–101.3%) for the determination of SUHs in grain samples.

4. Conclusion

In this study, a novel molecularly imprinted polymer was prepared using MCNTs as the support matrix via surface imprinting technique. The obtained MMIPs were characterized by TEM, FT-IR, XRD, BET method, elemental analyzer and VSM. The equilibrium data of MMIPs were described by Scatchard analysis. The adsorption kinetics suited the second-order equation mechanism. The prepared MMIPs had excellent specific recognition toward SUHs. Moreover, they combined the magnetic properties of Fe_3O_4 nanoparticles with the outstanding mechanical properties and high surface area of CNTs. It could be easily separated from the suspension by an external magnetic field, leading to fast and selective extraction of SUHs from grain samples. SUHs in extract were directly determined by HPLC. It is believed that this novel method coupling MMIPs extraction with HPLC detection can be one of the most promising candidates for rapid and selective analysis of complex matrixes.

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Figure captions:

Fig. 1. Polymerization process for MMIPs.

Fig. 2. The TEM image of CNTs (a) and MMIPs (b); FT-IR spectra of CNTs, MNIPs and MMIPs (c); The hysteresis loops of MMIPs (d); XRD patterns of Fe_3O_4 and MMIPs (e).

Fig. 3. Selections of the MMIPs amount (a); adsorption time (b); shaking rate (c); desorption solvent (d) and elution times (e).

Fig. 4. HPLC chromatograms of SUHs standard solution (a), blank soybean sample (b) and spiked soybean sample (c).

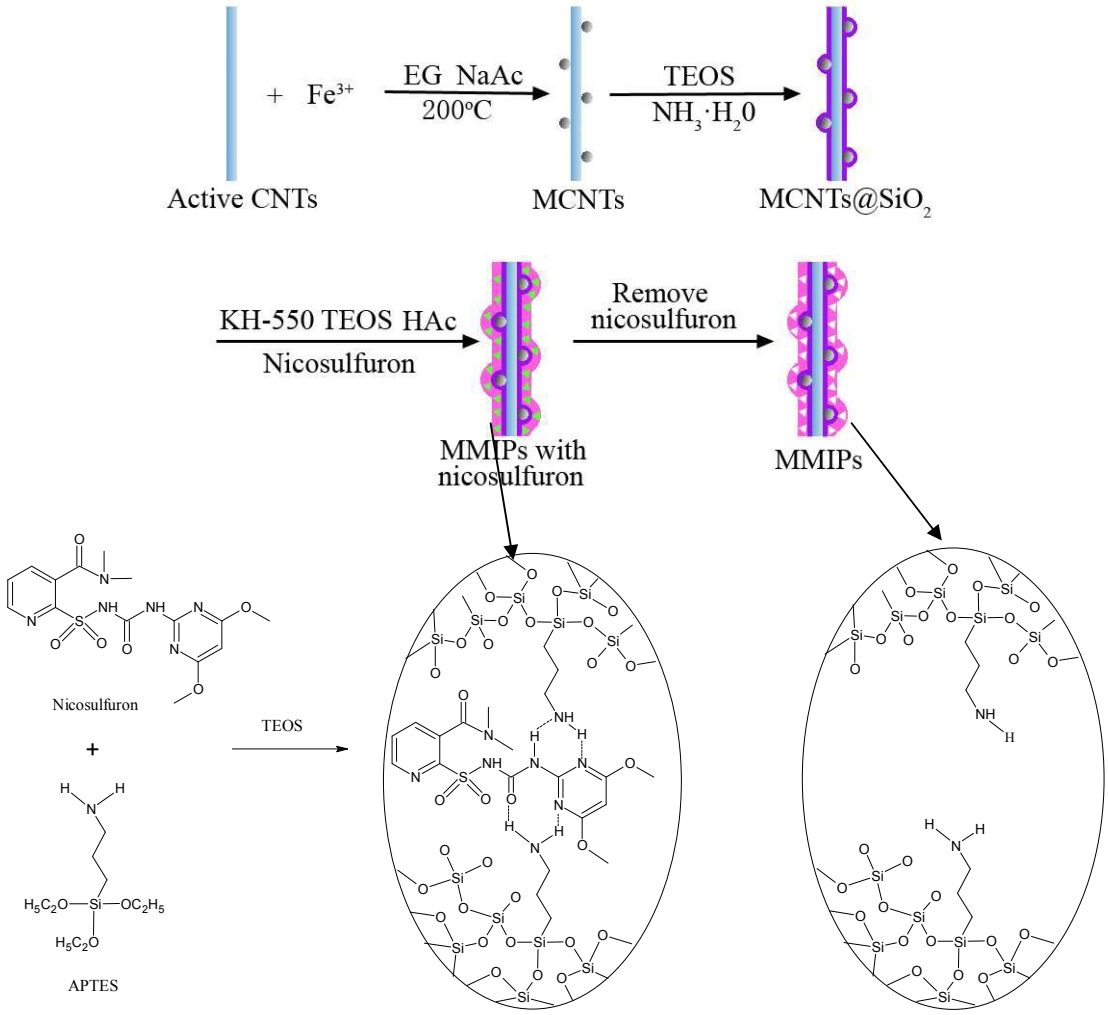


Fig. 1

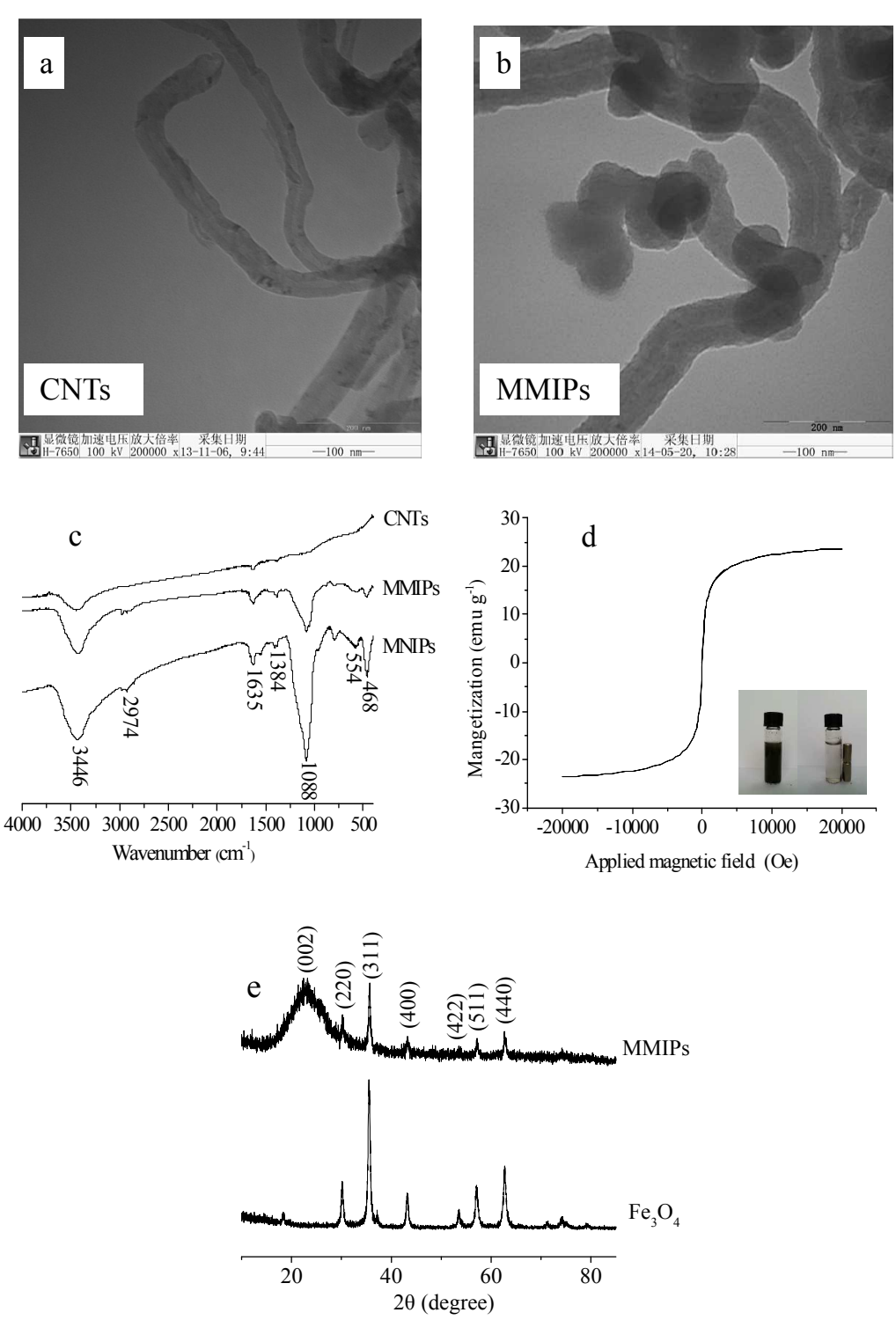


Fig. 2

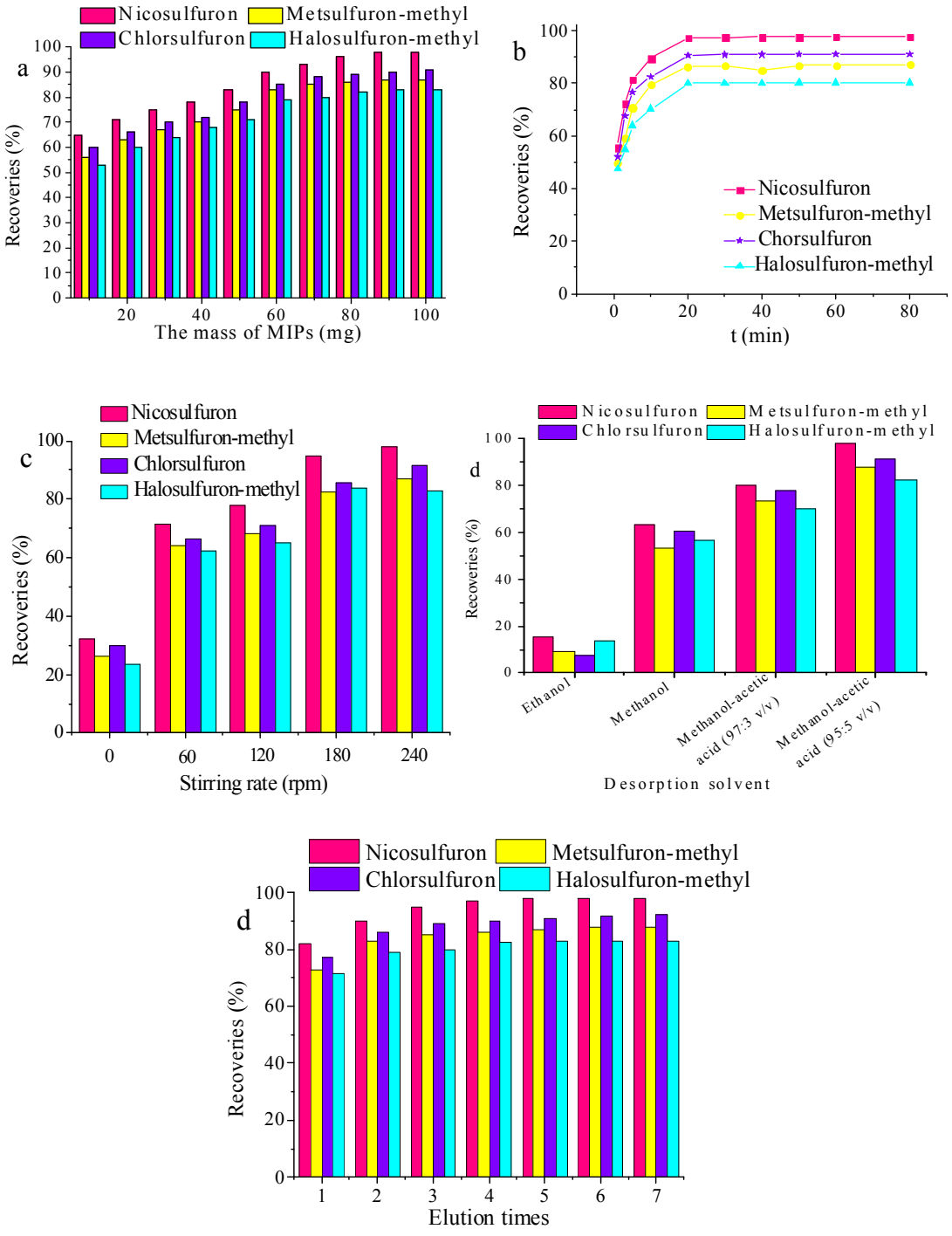


Fig. 3

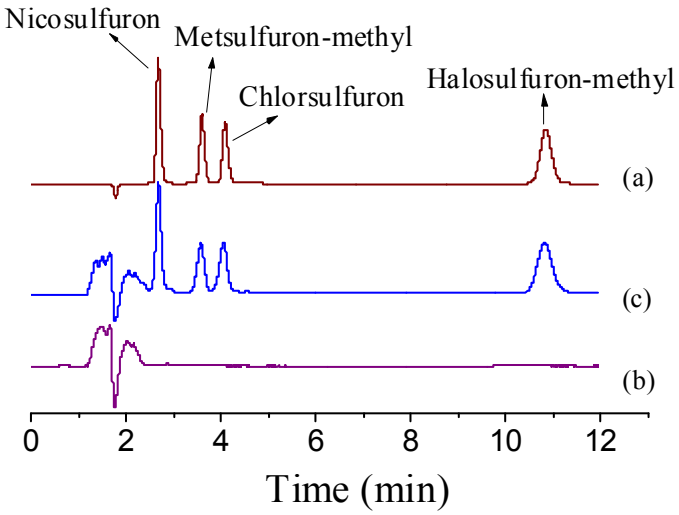


Fig. 4

Table 1 Kinetic Parameters for Adsorption of Nicosulfuron onto MMIPs and MNIPs.

Adsorption material	First-order				Second-order				Q_{eq}
	Equation	K_1	Q_{e1}	R^2	Equation	K_2	Q_{e2}	R^2	
MMIPs	$y=-9.443\times10^{-4}x+0.6165$	2.174×10^{-3}	4.14	0.2414	y=0.07629x+0.01522	0.3824	13.11	0.9999	13.09
MNIPs	$y=-4.472\times10^{-4}x+0.5901$	1.031×10^{-3}	3.89	0.3471	y=0.1314x+0.03238	0.5332	7.61	1.0000	7.61

Table 2 Selective Properties of MMIPs.

Analytes	MMIPs			MNIPs			K''
	Q	K_d	K'	Q	K_d	K'	
Nicosulfuron	47.50	0.095	3.33	22.52	0.045	1.67	2.00
Metsulfuron-methyl	43.10	0.086	3.02	21.12	0.042	1.56	1.94
Chlorsulfuron	45.07	0.090	3.16	22.09	0.044	1.63	1.94
Halosulfuron-methyl	40.51	0.081	2.84	21.53	0.043	1.60	1.78
Cyanazine	14.25	0.028	-	13.50	0.027	-	-

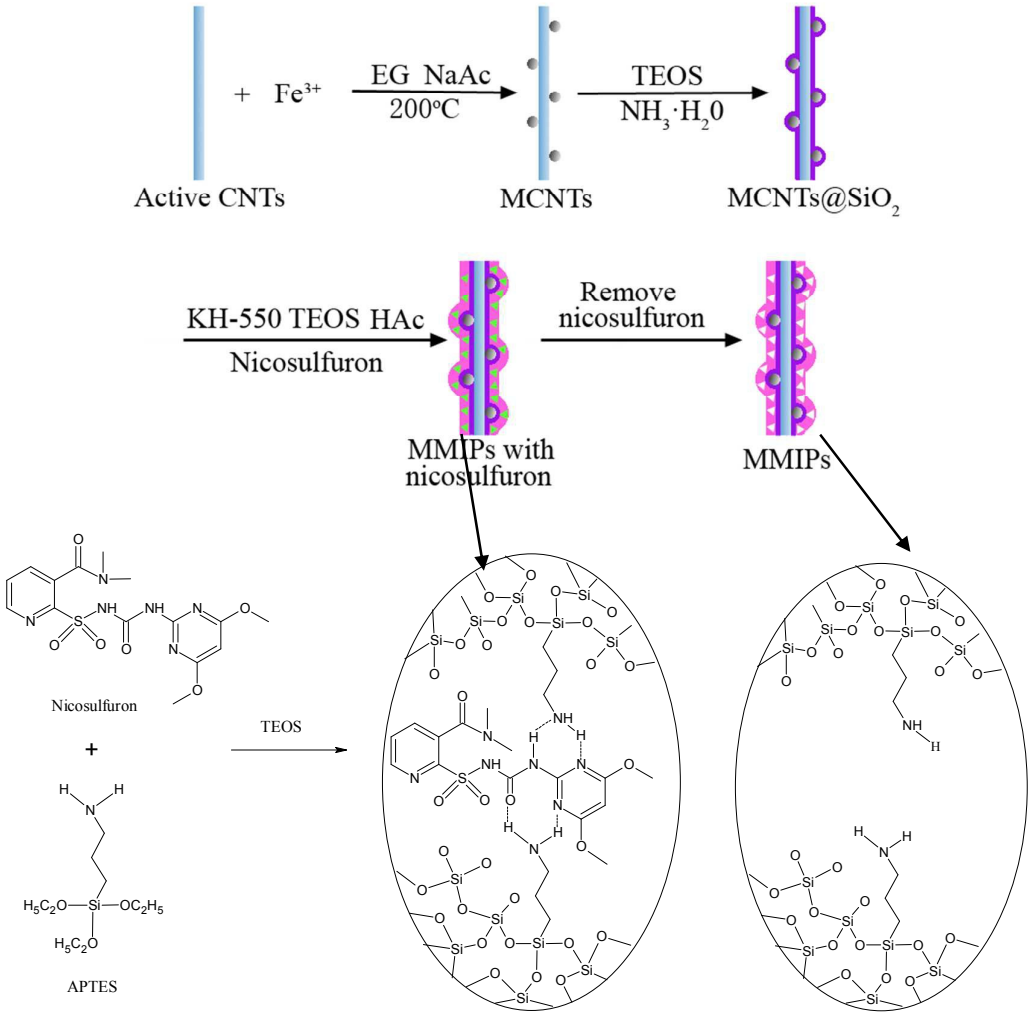
Table 3 The Linearity Range, Limit of Detection, Limit of Quantification and RSD of SUHs.

Analaytes	Liner range (mg kg^{-1})	LOD (mg kg^{-1})	LOQ (mg kg^{-1})	RSD (%)	
				Intra-day	Inter-day
Nicosulfuron	0.05-10	0.0107	0.0321	0.7	5.3
Metsulfuron-methyl	0.05-10	0.0151	0.0453	1.7	7.9
Chlorsulfuron	0.05-10	0.0147	0.0441	1.5	7.1
Halosulfuron-methyl	0.05-10	0.0123	0.0369	2.4	8.4

Table 4 Comparison of the Proposed Method with Other Methods Used in the Literatures.

Samples	Sample preparation method	Detection method	Liner range	Recoveries (%)	LOD	RSD (%)	References
Environmental water	Solid-phase extraction	LC-MS-MS	0.05-5000 ng L^{-1}	81.5-110.5	0.01-0.20 ng L^{-1}	1.9-7.4	3
Food crops	Matrix solid-phase dispersion	HPLC-VWD	0.20-10 mg kg^{-1}	62.0-102.6	0.02-0.07 mg kg^{-1}	<6.9	13
Fruit juices	Dispersive liquid–liquid microextraction	Capillary HPLC-DAD	8-100 μg L^{-1}	72.0-109.5	2-9 μg L^{-1}	1.0 - 9.8	39
Drinking water	Solid-phase extraction	LC-DAD	0.20-1.20 ng L^{-1}	84.0-107.0	2-16 ng L^{-1}	<20.0	40
Banana juice	Salting-out assisted liquid–liquid extraction	Capillary HPLC	12.50-125 μg L^{-1}	72.0-115.0	3-13 μg L^{-1}	1.2-9.9	41
Tea	Matrix solid-phase dispersion cleanup followed by dispersive liquid–liquid microextraction	HPLC-DAD	5.00-10000 mg kg^{-1}	72.8-110.6	1.31-2.81 mg kg^{-1}	＜7.0	32
Grains	Magnetic molecular imprinting technique	HPLC-UV	0.05-10 mg kg^{-1}	82.2-98.0	10.70-15.10 μg kg^{-1}	0.7-8.4	This work

Graphical Abstract



Polymerization process for magnetic molecularly imprinted polymers.