Molecularly Imprinted Poly(MAA-*co*-AM) Composite Membranes for Selective Recognition of Nicosulfuron Herbicide

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Received 28 January 2011; accepted 1 February 2012
DOI 10.1002/app.36938
Published online in Wiley Online Library (wileyonlinelibrary.com).

ABSTRACT: Molecularly imprinted membranes with different ratio of acrylamide (AM) versus methacrylic acid (MAA) were prepared by photocopolymerization on commercial filter paper using nicosulfuron as the template. The structures, the thermal stability, and the morphology of membranes were characterized by infrared spectroscopy (IR), thermogravimetric analysis (TGA), and scanning electron microscopy (SEM), respectively. Static equilibrium binding and competitive recognition properties of the membranes to nicosulfuron and its analogs (pyrazosulfuron ethyl and bensulfuron methyl) were tested. The results showed that nicosulfuron-imprinted membranes had the best recognition capacity to nicosulfuron compared with its analogs. The biggest selectivity factors of α_{N_1/P_2} and α_{N_1/B_3} were 1.28 and 1.83 and the imprinted factor reached to 2.34. The results of this study implied that the molecularly imprinted composite membranes could be used as separation membranes for nicosulfuron enrichment. The Scatchard plot revealed that one class of binding sites was mainly produced in the imprinted composite membrane in the studied concentration range of nicosulfuron.
 J Appl Polym Sci 000: 000–000, 2012

Key words: molecularly imprinted polymer; composite membrane; selective recognition; nicosulfuron; sulfonylurea herbicides

INTRODUCTION

Since introduced by Wulff and Sarhan in 1972,[1] molecularly imprinted technology has become a new technology with bright application prospect and significant research value. The molecular imprinting process is composed of three steps: first, functional monomer and template molecules interact by means of covalent or noncovalent bond. Second, the functional monomer template system is polymerized. Finally, template molecules are extracted from the polymer. So, many cavities are formed in the polymer where the template molecules occupied. These cavities ideally retain both the shape complementarity to the template and the strategic arrangement of the functional groups between the template and the functional monomer so as to turn into the specific recognition sites in the polymer.[2–4]

Owing to the current fascination with designable materials, much attention has been focused on the molecularly imprinted technology,[5–7] in which the noncovalent method, reported by Arshady and Mosbach in 1981,[8] relying on the weak interaction between the functional monomer and the template as well as the convenient operation has been paid more and more attention.[9–12] Molecularly imprinted membrane is a new research field in the last decades which develops along with the development of the molecular recognition polymer material. Nowadays, molecular imprinting technology has been applied to the preparation of molecular recognition polymer membrane that has specific function to identify small molecules.[13–18]

Nicosulfuron is the only sulfonylurea herbicide that has special effect to the gramineae weed so as to be used widely. Other names of nicosulfuron, including trade names, are Milagro, Sanson, Ghibli, Mistral, Accent, Nisshin, SL-950, MU-495 (Ishihara), DPX-V9360 (Dupont), and so on. The residue of this kind of herbicide in corn grain is stipulated by regulations in many countries at present.[19,20]

Because of the special functional groups in the nicosulfuron molecules, hydrogen bonds can be formed among different groups of nicosulfuron and the hydroxyl or carbonyl groups of methacrylic acid (MAA) and/or the carbonyl or amino groups of

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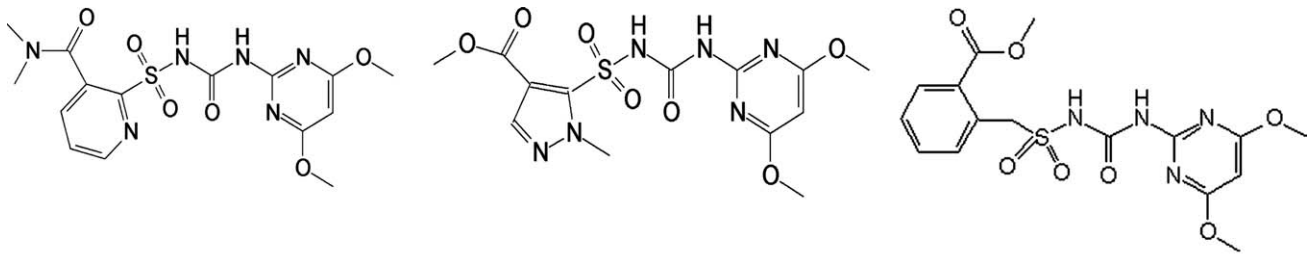
Contract grant sponsor: National Natural Science Foundation of China; contract grant number: 20972039.
Contract grant sponsor: Natural Science Foundation of Hebei Province; contract grant number: B2011201081.



Nicosulfuron (N_1) **Pyrazosulfuron ethyl (P_2)** **Bensulfuron methyl (B_3)**

Figure 1 Structures of nicosulfuron (N_1), pyrazosulfuron ethyl (P_2), and bensulfuron methyl (B_3).

acrylamide (AM). In this study, porous filter paper was used as support, AM and/or MAA as functional monomer and nicosulfuron as template molecular, molecularly imprinted composite membranes (MICM) and nonimprinted composite membranes (NICM) were prepared following the surface imprinting method by UV photocopolymerization. The recognition properties of MICM and NICM with different ratio of MAA and AM to different herbicides, including nicosulfuron and two analogs, and the optimum ratio of MAA and AM were investigated in this study.

Nicosulfuron-templated composite membranes using AM and/or MAA as functional monomer have not been reported so far. The structures, the thermal stability, and the morphology of membranes were characterized by infrared spectroscopy (IR), thermogravimetric analysis (TGA), and scanning electron microscopy (SEM), respectively. Static equilibrium binding and competitive recognition properties of the membranes to nicosulfuron (N_1) and its analogs (pyrazosulfuron ethyl (P_2) and bensulfuron methyl (B_3)) were tested. The results could provide the theory basis to the application of nicosulfuron-templated composite membranes to testing the residue of this kind of herbicide in crops, which is significant during the protection of food safety.

EXPERIMENTAL

Materials and reagents

Nicosulfuron (N_1), pyrazosulfuron ethyl (P_2), and bensulfuron methyl (B_3) were purchased from Xiangyu Pesticide Co., Ltd. (Nanjing, China). The molecular structures were shown in Figure 1. MAA, AM, ethylene glycol dimethacrylate (EGDMA), and 2,2′-azobisisobutyronitrile (AIBN) were purchased from Sigma-Aldrich (Shanghai, China). EGDMA and MAA were distilled to remove the inhibitors. AM was recrystallized with water before used. Microporous filter membrane (aperture: 1.2 μm) was bought from Automatic Science Instrument Co. (Tianjin, China) and was used as the membrane support. All the other chemicals were of the analytical or the high-performance liquid chromatography (HPLC) grade and used without further disposal. Doubly deionized water (DDW) was used throughout. Solutions for HPLC were filtered through a 0.45 μm membrane filter.

The stock solutions of N_1, P_2, and B_3 were prepared at a concentration of 0.08 g L^{-1} in acetonitrile (ACN) and diluted to the final concentration with ACN when used. All solutions were stored at −4°C in a refrigerator and reprepared every month.

Instrumentation and analytical conditions

The HPLC analysis of the samples in this study was performed on an HPLC system (American Agilent 1100 series) with UV–vis detector. The analysis of three sulfonylurea herbicides by HPLC was optimized according to the article.[21,22] The samples were performed at 240 nm on a XDB C18 column (5 μm, 250 × 4.6 mm) at the flow rate of 1.0 mL min^{-1}. The operated mobile phase was ACN : DDW (50 : 50), in which pH of water was adjusted to 2.5 with phosphoric acid. The injection volume of all the samples was 20 μL and the column temperature was 45°C.

The membranes after extraction and the template were characterized by IR spectroscopy analysis (FTS-40 spectrophotometer, BID-RAD, American). The TGA of MICM and that of the filter paper were performed on a Pyris6 apparatus (PerkinElmer, American). The morphology of the resultant polymer membranes was observed through a field emission SEM (KYKY-2800B, China).

Preparation of nicosulfuron molecularly imprinted polymer membranes

The membranes were prepared via photocopolymerization with microporous filter paper as membrane support between two even and transparent glass plates (20 cm × 10 cm). A total of 0.2 mmol of the template (N_1), free-radical initiator (AIBN), the monomer (AM and/or MAA), and the crosslinker (EGDMA) were dissolved in ACN in a 50 mL conical flask. The compositions of the reagents were shown in Table I. After sonicated for 15 min and

TABLE I
Composition of Poly (AM-*co*-MAA) Membranes

Membranes	Nicosulfuron (mmol)	MAA (mmol)	AM (mmol)	EDGMA (mmol)	AIBN (mg)
MIM1	0.2	1	0	5.0	10
MIM2	0.2	0.8	0.2	5.0	10
MIM3	0.2	0.6	0.4	5.0	10
MIM4	0.2	0.4	0.6	5.0	10
MIM5	0.2	0.2	0.8	5.0	10
MIM6	0.2	0	1	5.0	10
NIM1	–	1	0	5.0	10
NIM2	–	0.8	0.2	5.0	10
NIM3	–	0.6	0.4	5.0	10
NIM4	–	0.4	0.6	5.0	10
NIM5	–	0.2	0.8	5.0	10
NIM6	–	0	1	5.0	10

deoxygenated with nitrogen for 5 min, the reagent was spread on the microporous membrane. Then the microporous membrane was deposited between two glass plates immediately. The air bubble between the glass plates and microporous membrane was carefully removed by placing a constant weight on the glass plates. The polymerization was performed at 30°C under a 1000 W UV lamp at 365 nm for 12 h. The composite membranes were intensively extracted with ACN : acetic acid (9 : 1) for 6 h first after the polymerization completed to remove the unreacted polymerization reagents and N_1 from the membranes and then extracted with methanol for 2 h to remove rest acetic acid. The membranes were preserved in ACN for 12 h and were dried to constant weight for the future usage. NICM were prepared according to the same procedure, in which N_1 was not added in the reaction system.

Analysis of FTIR, SEM, and TGA

The MICM and the NICM prepared according to the process mentioned above were placed directly on the ATR crystal and firmly fastened by clips. The infrared spectra of MICM (MAA : AM = 0 : 1, 1 : 0, 2 : 3), NICM (MAA : AM = 1 : 0), and N_1 were obtained by FTS-40 Fourier infrared spectrometer.

The morphology of the resultant polymer membrane samples was observed through KYKY-2800B SEM. The cross-sectional view of the membranes for the SEM was prepared in liquid nitrogen and breaking it to produce a cross section. All the samples were sputtered coater with gold. The TGA of MICM after dried and extracted (MAA : AM = 2 : 3) and that of the filter paper were performed on Pyris6 apparatus in atmospheric oxygen at a heating rate of 10°C min^{-1}.

Adsorption experiments of N_1 on the imprinted membranes

Binding properties of MICM and NICM to three sulfonylurea herbicides were studied by the static equilibrium binding experiment. Nicosulfuron-imprinted and nonimprinted membranes were weighted (about 0.16 g) and then equilibrated with 5 mL of 0.08 g L^{-1} N_1 solution for 12 h at 20°C. The final volume of the solution was fixed to 10 mL with ACN and then the concentration was estimated by HPLC. The equilibrium adsorption capacity of N_1 (Q, μg g^{-1}) were calculated according to eq. (1)[23]: $Q = V(C_0 - C_i)/m$, where V is the volume of solution (mL); C_0 and C_i is the initial and balanceable concentration of the sulfonylurea herbicides in ACN solution (mg L^{-1}), respectively. The binding capacity of MICM and NICM to P_2 or B_3 was performed according to the same procedure.

To investigate the binding performance to N_1 of the MICM, binding isotherm was determined in the 0.5 to 5.0 mmol L^{-1} range of N_1. A total of 0.04 g MICM was placed into a conical flask and mixed with 3.0 mL of a known concentration of substrate. The flask was oscillated in a constant temperature bath oscillator at 20°C for 12 h. The concentration of free substrate (C_e) in the solution was determined using HPLC.[24] The amount of substrate bound to the membrane was calculated according to eq. (1). The obtained data were plotted according to the Scatchard eq. (2)[25]: $Q/C_e = (Q_{max} - Q)/K_d$, where K_d is the equilibrium dissociation constant and Q_{max}, an apparent maximum number of binding sites.

To evaluate the competitive recognition properties of MICM, MICM and NICM with different ratio of MAA and AM were weighted and then equilibrated in 5 mL 0.08 g L^{-1} of the sulfonylurea herbicides solutions for 12 h at 20°C. The final volume of the solution was fixed to 10 mL with ACN. The concentration was estimated by HPLC. The equilibrium adsorption capacity of sulfonylurea herbicides bound on the membranes was calculated according to eq. (1). The selectivity factor (α) was calculated as the ratio between the amount of template and the amount of the template analog bound on the membranes as eq. (3)[26]: $\alpha_{t/a} = Q_t/Q_a$, where Q_t (μg g^{-1})

is the template amount bound on the membrane and Q_a (μg g^{-1}) is the analog amount bound on the membrane. The imprinted factor (IF) was determined as eq. (4): $IF = K_{MICM}/K_{NICM} = (Q_{MICM}/C_{freeMICM})/(Q_{NICM}/C_{freeNICM})$.

RESULTS AND DISCUSSION

Optimization of experimental conditions and studies of recognition mechanism

To optimize the experimental conditions, a variety of factors such as reaction solvent, extraction solvent, extraction time, equilibrium time impacting on the results were evaluated.

Determination of reaction solvent

In polymerization, N_1 and its analogs should be dissolved in reactant mixture firstly and then be spread on the filter paper and glass plates. Therefore, the solubility of N_1 in the solvent and the workability of the solution were the key of reaction process. Therefore, the relevant experiments to different solvent systems were performed respectively.

The results showed that the solubility of N_1 and its analogs in dichloromethane and dichloromethane: dimethylformamide (4 : 1) was very well. But the volatility of the two kinds of solvent is high so that the desired membranes were not able to be prepared perfectly. N_1 and its analogs were dissolved in methanol, tetrahydrofuran or ACN : DDW (1 : 1) difficultly. However, in the solvent ACN, N_1 and its analogs was dissolved sufficiently and the reaction could be performed smoothly. Finally, ACN was chosen as the solvent in the study.

Studies of extraction solvent and extraction time

To eliminate N_1 in MICM, several kinds of extraction solvent, such as methanol : acetic acid (9 : 1), ACN : acetic acid (4 : 1, 9 : 1), were investigated. The results showed ACN : acetic acid (9 : 1) was economical and effective in the extraction of nicosulfuron.

The extraction time (3 h, 6 h, 9 h, 12 h) with ACN : acetic acid (9 : 1) as extraction solvent was researched also. After extracted with ACN : acetic acid (9 : 1) and then with methanol for 2 h, membranes were preserved in ACN for 12 h so as to ensure all template molecules could be extracted completely. The result showed the extraction time of 6 h was sufficient and fit.

Adsorption equilibrium time

The influence of the equilibrium time (3, 6, 9, 12, 24, and 48 h) on the adsorption capacity was investigated. At the initial stage from the beginning to 12 h, the binding amount of nicosulfuron on membranes rapidly increased along with the equilibrium time extension. Then the binding amount increased slowly after 12 h, so further extending the equilibrium time was largely meaningless. It was presumed that the binding amount of nicosulfuron on membranes reached saturated after 12 h. Therefore, the equilibrium time 12 h was determined in both binding experiments and selectivity experiments.

Studies of recognition mechanism

In N_1 molecules, sulfonyl oxygen, carbonyl oxygen, and heterocyclic ring nitrogen are able to form many hydrogen bonds with hydroxyl group or amino group of MAA and AM easily because of their free electron pairs. Amino hydrogen of sulfonylurea maybe also attract carbonyl oxygen of MAA and AM to form hydrogen bonds. Lots of hydrogen bonds made nicosulfuron molecules stationary in the reaction system and then special cavities were formed after extraction. A representative scheme for the formation of nicosulfuron molecularly imprinted membranes and the recognition mechanism of MICM were presumed and reported in Figure 2.

Characteristic of the FTIR spectra, TGA, and SEM analysis

Analysis of the FTIR

Although the copolymerization finished, a thin and transparent layer of copolymer was coated on the microporous support. FTIR spectra of N_1, NICM, and MICM (MAA : AM = 0 : 1, 2 : 3, 1 : 0) after the templates extracted were performed and shown in Figure 3; so that the polymerization and the binding mechanism between the imprinting sites and the template molecules were confirmed.

The main characteristics observed were carbonyl stretching vibration absorption bands at 1731.77 cm^{-1} in the IR spectra, corresponding to the carbonyl groups of PMAA, PAM, and P (MAA-*co*-AM) copolymer chains and nicosulfuron. It was found that the intensity of O—H stretching vibration absorption bands of MICM (b) and (d) spectra at 3581.18 cm^{-1} was lower than that of the NICM (e). The O—H stretching vibration absorption bands results from MAA in the membranes. Because nicosulfuron molecules could interact with hydroxyl groups of MAA and occupy a certain space in MICM (b) and (d) during the polymerization, this implied that there were many cavities generated after the template molecules were extracted, leading to the decreasing of hydroxyl groups in MICM. At the same time, it was also found that the stretching absorption bands of free NH_2 groups in (c) and (d) fell at 3480.90 cm^{-1}, which was different from those

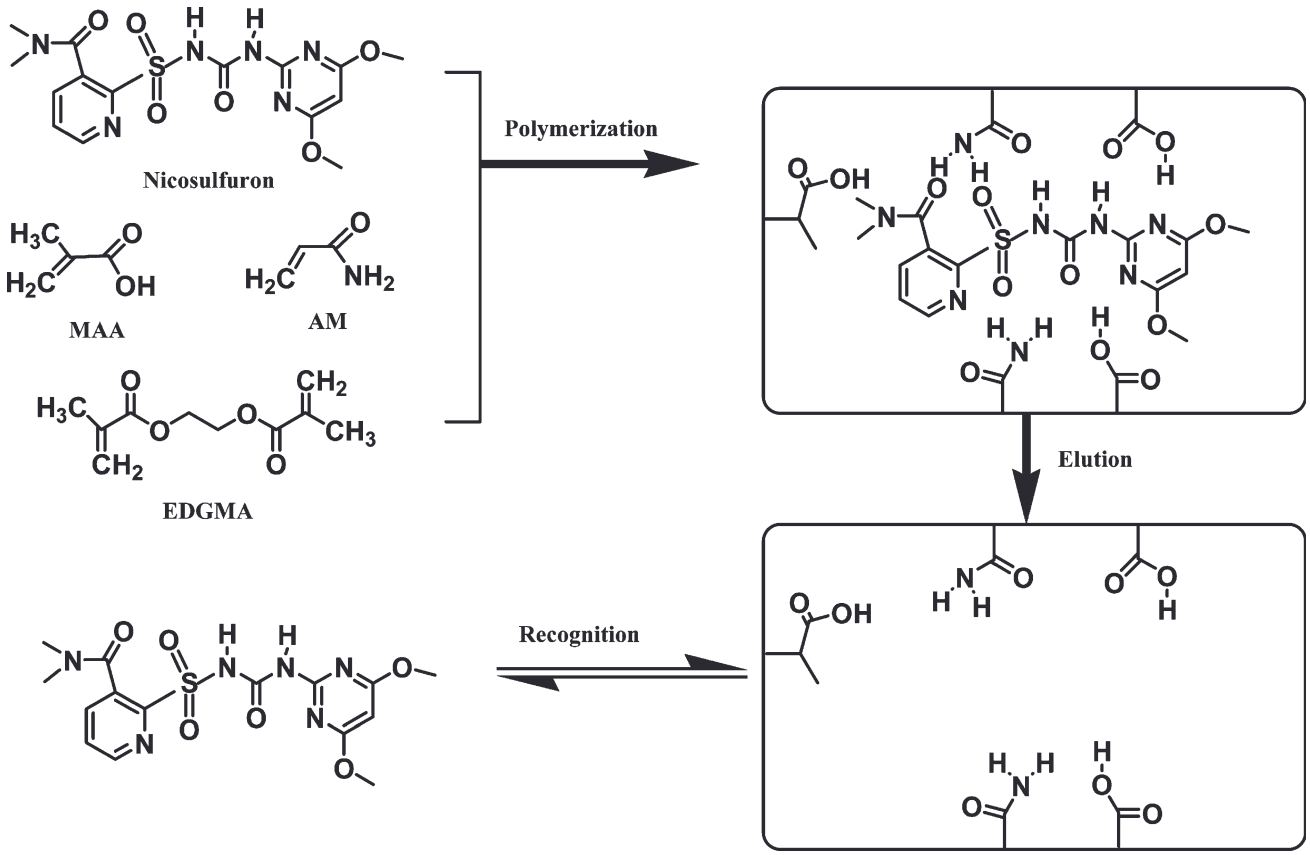


Figure 2 The formation and the recognition mechanism of nicosulfuron molecularly imprinted polymer membranes.

in (b) and (e). Similarly, the intensity of NH_2 stretching vibration absorption band of (c) was higher than that of (d). Therefore, it was concluded that P (MAA-*co*-AM) membranes and massive cavities were produced during the preparation of MICM. In these cavities, the interactions between the functional sites of the copolymers and the templates were formed via hydrogen bonding of the free COOH groups and $CONH_2$ groups.

Moreover, the absorption band at 1461.79 cm^{-1} was attributed to the symmetric deformation of methylene groups in the polymer backbone of membranes. The peaks in the region of 1567.85 and 1658.49 cm^{-1} were characterized the stretching vibration of C=N and C=C in pyridine and pyrimidine rings of nicosulfuron molecules which did not appear in the spectra of membranes. Consequently, all nicosulfuron molecules could be extracted completely in the process mentioned above.

Analysis of the TGA

TGA of filter paper and that of the imprinted membranes was shown in Figure 4. An obvious difference was found between them. The TGA of filter paper (a) showed a weight loss in two stages. At the first stage between 26 and 110°C, it showed about 0.18% loss in weight. This corresponded to the loss of adsorbed and bound water. Then the quick weight loss started at 364°C and continued up to

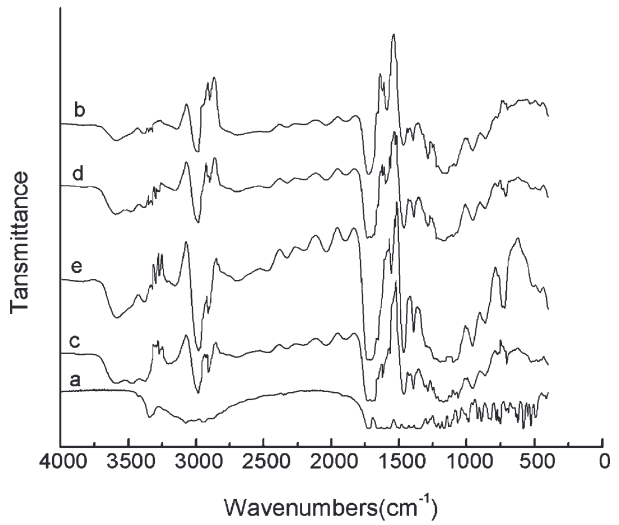


Figure 3 FTIR spectrum of nicosulfuron imprinted and nonimprinted membranes after extraction. (a) FTIR spectra of nicosulfuron, (b) nicosulfuron-imprinted membranes (MAA : AM = 1 : 0), (c) nicosulfuron-imprinted membranes (MAA : AM = 0 : 1), (d) nicosulfuron-imprinted membranes (MAA : AM = 2 : 3), and (e) nonimprinted membranes (MAA : AM = 1 : 0).

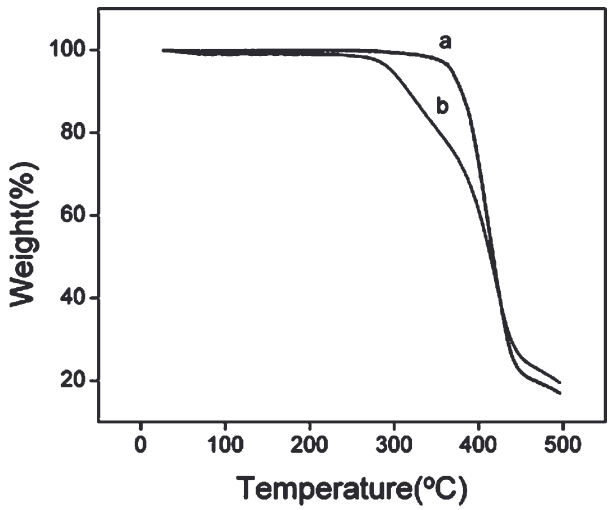


Figure 4 TGA of filter paper (a) and imprinted membranes after extraction (MAA : AM = 2 : 3) (b).

446°C, during which there was 72.89% of weight loss resulted from the degradation of the sole component of filter paper. However, the TGA of imprinted membranes after extraction (MAA: AM = 2 : 3) (b) was different and three stages of distinct weight loss between 26 and 620°C were observed. The first stage ranged between 26 and 110°C with 0.82% of the adsorbed and bound water weight loss. The second stage of weight loss started at 285°C and continued up to 382°C, during which there was 25.84% of weight loss because of the degradation of P (MAA-*co*-AM). There was 47.62% weight loss in the third stage from 382 to 465°C that contributed to the decomposition of the filter paper. So, it was evident that imprinted membranes were generated onto filter paper and could change the stability of filter paper at higher temperature.

Analysis of the SEM

SEM images of the imprinted and nonimprinted membranes and the filter paper were shown in Figure 5. From the images, it was clearly indicated that there were small and innate holes in the filter paper (a). The NICM were smoother and more compact (b). Many uniform gaps were in NICM, which was because of the existing of ACN at the time of

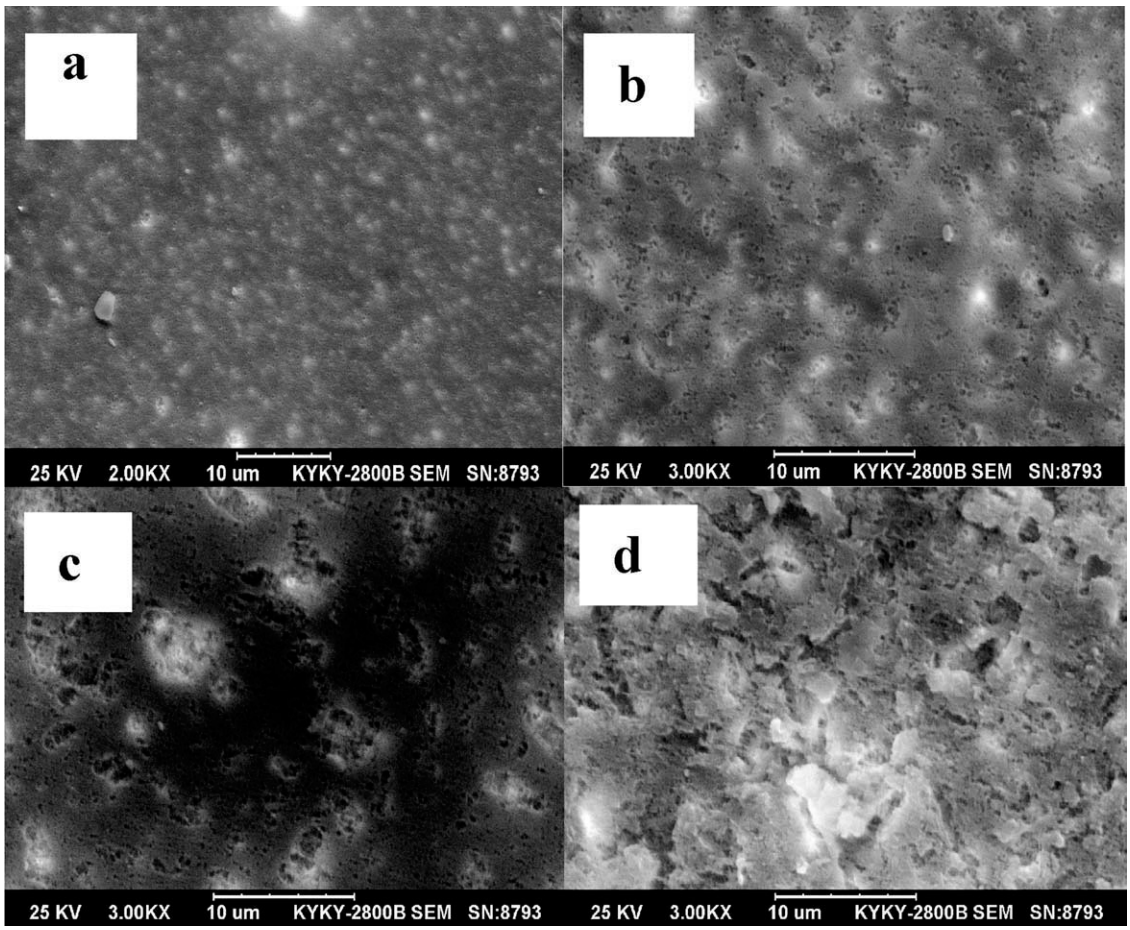


Figure 5 SEM images of filter paper and membranes after extraction: (a) the surface image of the filter paper, (b) the surface image of NIM, (c) the surface image of MIM (MAA : AM = 2 : 3), and (d) the surface image of MIM (MAA : AM = 1 : 0).

polymerization and the removing of ACN after the extraction. The figures (c) and (d) showed special scene. The MICM surface of PMAA (d) was much rougher than the other membranes, but it was clear that there were bigger cavities in (c) and (d). The special cavities might derive from the hydrogen bond interaction between the template molecules and the functional groups of MAA and AM. Before the polymerization, the template molecules interacted with functional monomers to form complex mixture system, in which the template molecules were fastened and monomers turned into special sequence structure and special frame pattern. During the polymerization, monomers were initiated and polymerized with template molecules distributed in this specific system. Finally, hydrogen bonds among template molecules and MICM were destroyed and then the template molecules were removed as MICM were extracted by ACN : acetic acid (9 : 1). MICM with lots of special cavities were produced at last.

Effect of functional monomers on binding capacity of MICM and NICM

To plot the calibration curves, standard solutions of N_1, P_2, and B_3 (concentrations between 2.0 and 40.0 mg L^{-1}) were prepared. Twenty microliters of each of the standard solutions was injected into the HPLC under the chromatography conditions described above. The linear equations of nicosulfuron, bensulfuron methyl, and pyrazosulfuron ethyl were $y = 50.616x + 16.72$ ($R^2 = 0.9999$); $y = 70.457x - 2.72$ ($R^2 = 0.9998$); $y = 52.981x - 2.9249$ ($R^2 = 0.9996$), in which y and x represented adsorption peak area and the concentration (mg L^{-1}), respectively.

The results of binding properties of MICM and NICM to N_1, P_2, and B_3 were shown in Figure 6. Each binding amount was tested five times, and all the data were presented as average and the standard deviation ranged from 3.35 to 15.28. The membranes were weighted and compared with the weight of membranes last time. The recovery of membranes was 99.87 to 100.2%.

The amounts of N_1 bound by MICM (MICM-N_1) increased as the ratio of MAA and AM climbing up to 4 : 1, 3 : 2, and 2 : 3. The best binding capacity (363.21 μg g-membrane^{-1}) was the membrane in which MAA : AM was 4 : 1. All the binding amounts of MICM were more than 250.43 μg g-membrane^{-1}, which were higher than those of the NICM (NICM-N_1).

MICM also showed better binding capacity to P_2 than NICM (NICM-P_2). The amounts of P_2 bound by MICM (MICM-P_2) were all more than 149.47 μg g-membrane^{-1}. The membrane with the best binding

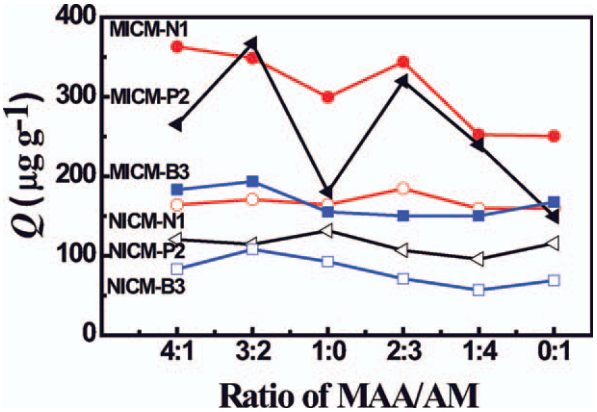


Figure 6 Effect of functional monomers on binding capacity of MICM and NICM. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

capacity (366.96 μg g-membrane^{-1}) was the MICM in which the ratio of MAA and AM was 3 : 2. As a whole, the values of MICM-P_2 and NICM-P_2 were less than those of MICM-N_1 and NICM-N_1 slightly.

The MICM with MAA : AM (3 : 2) showed the best binding capacity (193.40 μg g-membrane^{-1}) to B_3 (MICM-B_3), which was similar with the result of MICM bound to P_3. The amounts of B_3 bound to MICM were all more than 150.20 μg g-membrane^{-1}. But it was clear that the binding capacity of the membranes to B_3 was lower than that of membranes to N_1 and P_2 clearly. The amounts of B_3 bound to NICM (NICM-B_3) were the lowest among three compounds and changed slightly.

From the results obtained, it was found that the template-imprinted polymers had the best binding capacity to the template and P_2 had bigger binding capacity to MICM more than B_3. The answer could be found by the analysis of the different molecular structure of three sulfonylurea herbicides. In N_1 molecule, there is a pyridine ring that contains one nitrogen atom. The pyridine ring could form hydrogen bond with hydroxyl group or amino group of MAA and AM. P_2 has an imidazolyl ring in the molecule, which could form hydrogen bonds too. Therefore they had the bigger binding capacity to MICM and NICM. B_3 had the least binding capacity among them. This may be because it has a benzene ring at the corresponding position in the molecule that cannot form hydrogen bond. Moreover, the molecular structure of B_3 is bigger than that of N_1 or P_2, which may be another reason why B_3 molecules inserted the cavities more difficultly. Although the size of P_2 molecule is smaller than that of N_1 and could easily insert the specific cavities, P_2 molecules did not fit the binding position as correctly as N_1 so that it could not link with the main chains of copolymer as firm as N_1. Therefore, N_1 was the fittest to the binding cavities that were the specific recognition sites of

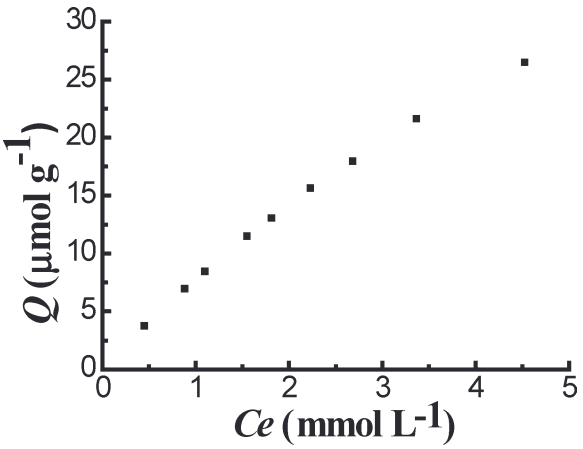


Figure 7 Binding isotherm of MICM.

MICM. When the binding process achieved equilibrium, the specific binding sites were occupied by nicosulfuron mainly.

Besides, MICM with the most adsorption amount were copolymer with certain ratio of MAA and AM. This may be because amide group has stronger hydrogen bonding ability in polar solvents and the monomer MAA can form intramolecular hydrogen bond.[27,28] Along with the increasing of AM, the amounts of the effective hydrogen bond that could form specific recognition sites increased. N_1, P_2, and B_3 have similar and special structures and could form hydrogen bond with both AM and MAA. But because of their structure characteristics mentioned above and the more advantage of COOH, they were more inclined to be adsorbed in the MICM with the higher ratio of MAA. Therefore, it could be found that there was a best proportion of MAA and AM in the MICM (4 : 1 or 3 : 2) that had the biggest binding capacity.

The results also showed that the binding capacity of the NICM to the three sulfonylurea herbicides were lower with little change. This indicated that the MICM with special cavities interacted more easily with template and its analogs than normal membranes. Moreover, because the surface of NICM was smoother and more compact than that of MICM and there were not fit cavities in NICM to the template and its analogs so that the interaction between NICM and samples limited on the surface of NICM mainly. Therefore, it was concluded that the NICM had little binding capacity to these herbicides compared with MICM.

Binding characteristics of MICM

To investigate the binding performance for N_1 in the resultant MICM (MAA : AM = 4 : 1), we determined the binding isotherm (Fig. 7) in the 0.5 to 5.0 mmol L^{-1} range of N_1. The obtained data were plotted according to the Scatchard equation as shown in Figure 8.

It was found that the Scatchard plot was linear indicating that the binding sites in MICM are uniform with respect to the affinity for N_1. Because there was one distinct section within the plot which can be regarded as straight line, it revealed that one class of binding sites was mainly produced in MICM in the studied concentration range of N_1. The equilibrium dissociation constant K_d and the apparent maximum number Q_{max} can be calculated to be 9.55 mmol L^{-1} and 82.50 μmol g^{-1} of dry MICM from slope and intercept of its Scatchard plot.

Competitive recognition properties of MICM

The competitive binding experiment was also performed in this study to test the selective capacity of MICM. The results of the experiment were shown in Table II. It was found that the amounts of N_1, P_2, and B_3 bound on the imprinted composite membranes were more than 237.3 μg g-membrane^{-1}, 215.6 μg g-membrane^{-1}, and 156.1 μg g-membrane^{-1}, respectively. The biggest selectivity factor of α_{N_1/P_2} (1.28) was obtained from the imprinted composite membrane with MAA : AM (4 : 1) and the biggest selectivity factor of α_{N_1/B_3} (1.83) was obtained from the imprinted membrane with MAA: AM of 2 : 3. According to the Table, all the selectivity factors were more than 1.09 and the selectivity factors α_{N_1/B_3} were larger than the selectivity factors α_{N_1/P_2} . Therefore, it could be concluded that the interaction of MICM with N_1 had the highest selectivity and competitiveness and P_2 had the binding capacity more than B_3.

The amounts bound by membranes in the competitive binding experiment were bigger than those in binding properties of the membranes as a whole. This may be because the three sulfonylurea herbicides had synergism in the same adsorption system so that the amounts bound by membranes in the competitive binding experiment increased.

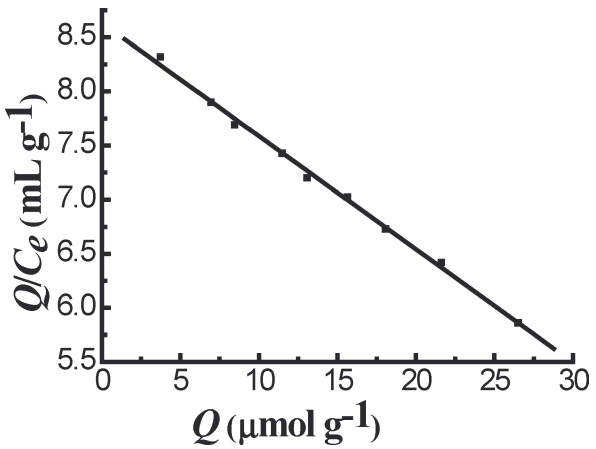


Figure 8 Scatchard plot to estimate the binding nature of MICM.

TABLE II
The Selectivity Factor and Imprinted Factor of MICM

	Q_{N_1} (μg g^{-1})		Q_{P_2} (μg g^{-1})		Q_{B_3} (μg g^{-1})				
MAA:AM	MIM	NIM	MIM	NIM	MIM	NIM	α_{N_1/P_2}	α_{N_1/B_3}	IF_{N_1}
1 : 0	442.8	210.6	374.9	129.8	303.3	83.8	1.18	1.46	2.34
4 : 1	560.1	273.8	438.8	152.8	355.8	122.4	1.28	1.57	2.32
3 : 2	366.6	275.7	335.4	149.3	287.3	79.9	1.09	1.28	1.43
2 : 3	308.4	275.0	254.9	57.8	168.9	17.5	1.21	1.83	1.16
1 : 4	291.5	263.2	238.6	109.6	172.6	81.3	1.22	1.69	1.14
0 : 1	237.3	163.9	215.6	122.5	156.1	21.4	1.10	1.52	1.78

Moreover, the samples with the higher concentration (total concentration of N_1, P_2, and B_3) have the bigger driving force to diffuse into the inner parts of the composite membranes. Therefore, the binding happened both on the surface and in the inner parts of the membranes in the competitive binding experiment. However, the driving force of the samples in binding properties experiment was not as big as that in the competitive binding experiment. The binding of N_1, P_2, and B_3 were mainly happened on the surface of the membranes and fewer molecules could diffuse into the inner parts of the membranes.[18]

According to the Table II, the biggest binding capacity of N_1, P_2, and B_3 appeared at the MICM with the MAA: AM of 4 : 1, which maybe result from the binding advantage of N_1 and the interaction among three compounds. But because the binding capacity of N_1 increased much more than those of P_2 and B_3, α_{N_1/P_2} and α_{N_1/B_3} were 1.28 and 1.57, and the selectivity factor of α_{N_1/P_2} (1.28) reached the biggest. Along with the increasing of the ratio of AM, the binding capacity of three compounds decreased gradually. When the ratio of MAA and AM reached 2 : 3, the binding capability of N_1 remained higher level but that of B_3 decreased sharply. This may be because the binding of N_1 was specific and that of B_3 was not specific and firm enough. The selectivity factor of α_{N_1/B_3} reached the biggest 1.83.

In the Table II, IF_{N_1} showed the selectivity of the imprinted and nonimprinted membranes in the mixed solution. It was clear that all of IF_{N_1} were more than 1.14 and the biggest value reached to 2.34 and 2.32 (MAA : AM = 1 : 0; 4 : 1). The results also revealed that MICM had very high binding selectivity for its template molecule in the mixed solution. Therefore, such copolymer membranes can concentrate the target molecules and then separate the molecules from the mixture solution with higher efficiency.

CONCLUSIONS

In this study, novel composite nicosulfuron-imprinted membranes with different ratio of MAA versus AM were prepared via photocopolymerization on filter paper using nicosulfuron as the template molecule. Several main experimental conditions of membranes were optimized. The results of binding experiments showed that MICM had the best binding capacity to N_1 (MAA : AM = 4 : 1) and also had certain binding capacity to its analogs.

Competitive binding experiments of MICM to N_1, P_2, and B_3 showed that MICM had the best recognition capacity to N_1 compared with its analogs in the mixed solution. The biggest selectivity factor of α_{N_1/P_2} was 1.28 and that of α_{N_1/B_3} was 1.83. They were obtained from the composite membranes in which the ratios of MAA and AM were 4 : 1 and 2 : 3, respectively. The imprinted factors with respect to MICM and NICM reached to 2.34. The results of this study implied that MICM could be used as separation membranes for nicosulfuron enrichment.

The Scatchard plot was presented as a straight line and it revealed that one class of binding sites was mainly produced in MICM in the studied concentration range from 0.5 to 5.0 mmol L^{-1} of N_1. The equilibrium dissociation constant K_d and the apparent maximum number Q_{max} were calculated to be 9.55 mmol L^{-1} and 82.50 μmol g^{-1}.

The structures and the thermal stability of membranes were characterized by IR and TGA. The morphology of the resultant membranes was visualized by SEM. The Figures showed that the polymer membranes were prepared on the filter paper and the morphology of MICM was different from those of NICM and the filter paper obviously. The thermal stability of MICM was higher than that of filter paper at higher temperature. According to the results, the possible interactions between the template and the imprinted composite membranes were suggested at last, in which part of COOH groups and $CONH_2$ groups of membranes participated in the linkage. The application research of MICM in the field of food safety detection is being performed.

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