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Preparation of a bifunctional pyrazosulfuron-ethyl imprinted polymer with hydrophilic external layers by reversible addition–fragmentation chain transfer polymerization and its application in the sulfonylurea residue analysis

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ABSTRACT

A new bifunctional pyrazosulfuron-ethyl imprinted polymer was synthesized by the combination of molecular imprinting technology and living radical polymerization. In the synthesis, the pyrazosulfuron-ethyl imprinted polymer was obtained by the reversible addition–fragmentation chain transfer (RAFT) precipitation polymerization followed by grafting poly(glyceryl monomethacrylate) (pGMMA) by the post-RAFT polymerization. In this research, we used polyethylene glycol (PEG) as the polymeric porogen in order to increase the porosity of the material which is a new porogen application in the precipitation polymerization. The imprinted polymer has selectivity for the template and ability of humic acids exclusion which has shown the merits of molecularly imprinted polymers and restricted access materials. An online solid-phase extraction/HPLC method for the analysis of three sulfonylurea residues in soil samples has been developed and validated. The recovery of 81–99% in the spiked levels of 40–200 μg kg^{-1} was obtained and the limit of detection (LOD) and limit of quantification (LOQ) were less than 4.8 and 15.9 μg kg^{-1} respectively. The results demonstrated that this bifunctional material can be used for the efficient pyrazosulfuron-ethyl extraction in the sulfonylurea residue analysis from environmental samples.

1. Introduction

Molecular imprinting is a technique of making molecularly imprinted polymer (MIP), an artificial material with enzyme-like property. MIP has attracted great attention in the analytical research due to the advantages such as pre-determined selectivity and chemical stability. Its application has involved in many analytical research areas such as sensors [1], chromatographic separation [2] and molecularly imprinted solid-phase extraction (MISPE) [3–5]. The MISPE has shown good potential in the complex sample analysis.

In the MIP synthesis, precipitation polymerization has been employed increasingly in recent years [6–8] due to its advantages in spherical particles preparation and free of interfering substances for the template-monomer interaction in the non-covalent imprinting process [9]. However, imprinted polymers from the precipitation polymerization often have low surface areas due to the specific chain propagation mechanism. Small surface areas can result in low binding capacities which is a drawback in the extraction applications. To increase pore volumes and surface areas of MIPs produced by the precipitation polymerization is one of the objects in this study.

Sulfonylurea herbicides are widely used pesticides due to the high activity and low toxicity to mammals. Because their application doses are low, determination of trace sulfonylurea residue is necessary in the environmental analysis. Different methods for the determination of sulfonylurea herbicides including HPLC [10], capillary electrophoresis (CE) [11], gas chromatography (GC) [12] have been established. In these methods, sample pretreatments are often employed which are generally time-consuming. Meanwhile, most sulfonylureas are acidic compounds. When they are extracted from the environmental samples, humic acids are often co-extracted and become interference in the HPLC analysis. In order to achieve an efficient sample pretreatment in the sulfonylurea analysis, MIPs have been synthesized and used for the

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selective sample extractions [13–18]. The bulk polymerization [13–15,18], surface grafting [16], one-step swelling [17] have been used in the MIP preparations. However, co-extracted humic acids are problems in the MISPE because large molecules in humic acids have more non-specific interactions with the MIP backbone chains.

To exclude humic acids in the chromatographic separation, restricted access material (RAM) has been developed [19]. RAM is a bifunctional material on which proteins and humic acids can be excluded by physical or chemical diffusion barriers, while small molecules are retained in the inner layers through hydrophobic or ionic interactions. In recent years, materials possessing both characters of RAM and MIP (RAM-MIP) have been developed [20–23]. The studies of using MIP-RAM in the environmental analysis have been published and its advantages have been demonstrated [24,25]. In this research, the poly(glycerol monomethacrylate), pGMMA, was grafted on the surface of the imprinted microspheres by the RAFT polymerization to establish a diffusion barrier (restricted access outer-layer) and prevents the humic acids from irreversible adsorption on the core MIP structure.

The reversible addition–fragmentation chain transfer (RAFT) polymerization is a controlled/living polymerization method. It can be employed to synthesize polymers with low polydispersity and to make block polymers with convenient procedure. RAFT has been used in the MIP synthesis in the preparation of microspheres [26,27], monolithic polymers [28,29] and grafting MIPs on the supports [30–32]. In this research, RAFT polymerization is used in the bifunctional MIP synthesis based on the following considerations. First, RAFT polymerization provides controlled chain propagation, which is in favor of making uniform polymeric particles. Second, RAFT can be employed in grafting pGMMA on the surface of imprinted polymer to form diffusion barriers for humic acids. In our previous work, a MIP material with hydrophilic out-layers has been prepared [23] by the RAFT precipitation and post-grafting polymerization. However, the MIP has very low surface area, which resulted in low binding capacity and weak retention of the template. In this research, The MIPs synthesized with different RAFT agent were compared. Meanwhile, polyethylene glycol (PEG) was employed as the porogenic agent in attempt to increase the porosity and the surface area of the imprinted polymers.

In this study, the synthetic conditions were optimized. To our best knowledge, it is the first report of using polymer as porogen in the precipitation polymerization for the MIP synthesis. The relations between the synthetic conditions and properties of the materials were investigated. An online SPE/HPLC method for the sulfonylurea residue analysis was developed and satisfied validation results were obtained.

2. Material and methods

2.1. Chemicals

Pyrazosulfuron-ethyl (PS), chlorosulfuron (CS), chlorimuron-ethyl (CME), monosulfuron (MS) and bensulfuron-methyl (BSM) were kindly provided by the National Pesticide Engineering Research Center (Tianjin, China). Methacrylic acid (MAA) was obtained from Damao Chemical Plant (Tianjin, China) and was distilled under vacuum before use. Ethylene dimethacrylate (EDMA) was from Yunkai Chemistry Corporation (Yantai, China) and was washed consecutively with 10% aqueous sodium hydroxide solution and water and then dried over $MgSO_4$ prior to use. 2,2′-Azobisisobutyronitrile (AIBN) and ammonium persulfate $[(NH_4)_2S_2O_8]$ were obtained from Shanghai Sihewei Chemical Co., Ltd. (Shanghai, China) and Tianjin Guangfu Fine Chemical Research Institute (Tianjin, China), respectively. Polyethylene glycol (PEG) was purchased from Chemical Experimental Plant of Tianjin University (Tianjin, China). The PEG-300, PEG-5000 and PEG-12,000 were used to represent polyethylene glycols with average molecular weight of 300, 5000 and 12,000 respectively. Glycerol monomethacrylate (GMMA) was from Meryer Chemical Technology Shanghai Company (Shanghai, China). Bovine serum albumin (BSA) was from Solarbio Science & Technology Co., Ltd. (Beijing, China) and humic acid sodium salt was obtained from J&K Chemical Ltd. (Beijing, China), respectively. All other chemicals were of analytical grade.

2.2. Preparation of the MIP microspheres by RAFT polymerization

To perform RAFT polymerization, two kinds of RAFT chain transfer agent (CTA): dibenzyltrithiocarbonate (DBTTC) and cumyl dithiobenzoate (CDB) were synthesized according to the literature [33,34]. The structures of the DBTTC and CDB are shown in Fig. 1.

The pyrazosulfuron-ethyl imprinted polymer was prepared via RAFT precipitation polymerization (RAFTPP) by the following procedure. In a conical flask, pyrazosulfuron-ethyl (imprinting molecule, 1 mmol) and MAA (functional monomer, 5 mmol) were dissolved in the porogenic agents. Acetonitrile, acetonitrile/toluene and PEG/acetonitrile were used as porogen in different experiments. EDMA (cross-linker), AIBN (initiator) and the chain transfer agent (DBTTC or CDB) were then added. The template/(functional monomer)/cross-linker was 1/5/25 (molar ratio). The molar ratio of monomers (MAA +EDMA)/AIBN/CTA was 300:1:4. Total monomers concentration in porogen was 3% (w/v). The solution was ultrasonicated to obtain a homogenous solution and deaerated with nitrogen. The flask was sealed and rotated horizontally in a water bath oscillator. The reaction temperature was increased from 20 to 60 °C in 40 min and then remained constant for 24 h. After the reaction, the particles were collected by centrifugation. The template and unreacted reagents in the product were removed by Soxhlet-extraction with MeOH/HOAc (8/2, v/v) and MeOH consecutively. The polymeric particles were then dried at 50 °C under vacuum. For comparison, non-imprinted polymer (NIP) was synthesized following the same procedure of the corresponding imprinted polymer except the absence of template.

2.3. Evaluation of binding characteristics of MIP by the Freundlich analysis

The binding affinity of the polymers was evaluated by the equilibrium adsorption experiment. In the experiment, polymeric particles (30 mg) were weighed and mixed with 3.0 mL of PS solutions with different concentrations. The concentrations of PS solutions were varied from 0.06 to 3.0 mmol L^{-1} with acetonitrile as the solvent. The mixture was shaken in a water bath oscillator for 24 h at room temperature and then centrifuged. The PS concentrations in the supernatants were determined by HPLC analysis using a Shimadzu LC-20A HPLC instrument (Shimadzu, Japan). The amount of PS bound to the polymer was calculated by subtracting the amounts of the free PS from the initial amount added to the mixture. The binding performance of the MIPs was

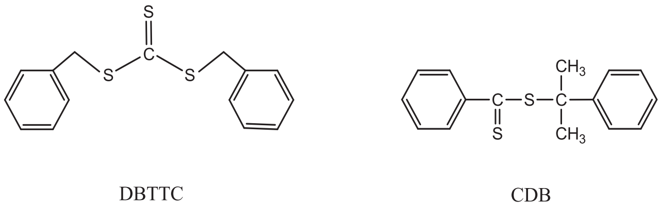


Fig. 1. RAFT chain transfer agents used in the study.

analyzed by the Freundlich isotherm equation [35]

$$B(F) = aF^m \tag{1}$$

where B and F are the concentrations of bound and free analyte, respectively. The constant m is the heterogeneity index.

By plotting the experimental binding data in log format, the fitting coefficients log a and the m were determined by the linear regression equation

$$\log B = m \log F + \log a \tag{2}$$

The total number of binding sites N_{K1-K2} in the concentration limits of the $F_{\min}$ and $F_{\max}$ was calculated with the following equation:

$$N_{K1-K2} = a(1-m^2)(K_1^{-m} - K_2^{-m}) \tag{3}$$

where, the affinity constant $K_1 = 1/F_{\max}$ and $K_2 = 1/F_{\min}$. The $F_{\min}$ and $F_{\max}$ are the minimum and maximum free PS concentrations. respectively.

The apparent weighted average affinity constant $\overline{K}$was calculated by the following equation:

$$\overline{K}_{K1-K2} = \left(\frac{m}{m-1}\right)\left(\frac{K_1^{1-m} - K_2^{1-m}}{K_1^{-m} - K_2^{-m}}\right) \tag{4}$$

Because binding isotherms variation rates are different in two concentration sections, the data from the initial concentration more than 500 mmol L^{-1} were used in the Freundlich analysis to obtain more reliable results.

2.4. Polymer characterization

The morphology of the polymers was observed by a scanning electron microscope (S-3500N, Hitachi, Japan). The porosities and surface areas were analyzed by the nitrogen sorption method on a Quantachrome NOVA 2000 instrument (Quantachrome Instruments, USA).

2.5. Determination of the selectivity of the MIPs by HPLC analysis

The microspheres were dry packed into a stainless steel HPLC column (50 × 4.6 mm). The chromatographic experiments were carried out with a Shimadzu LC-20A HPLC system. The measurements were performed at the room temperature and monitored at 240 nm. The retention factors (k) of the analytes were calculated as $k=(t_r-t_0)/t_0$, where t_r and t_0 are the retention time of the analytes and potassium dichromate, respectively. The selectivity factor (α) was calculated as $\alpha = k_p/k_x$, where k_p and k_x are the retention factors of pyrazosulfuron-ethyl and its analogs, respectively.

2.6. Grafting pGMMA chains on the surface of the MIP microspheres and evaluation

The RAFT polymerization for grafting pGMMA chains on the surface of MIP was carried out in a three-neck glass flask. The MIP particles (0.7 g) were suspended in a solution containing GMMA, $(NH_4)_2S_2O_8$ and H_2O (20 mL). Different GMMA concentrations were used. The GMMA/$(NH_4)_2S_2O_8$ was 100/1 (molar ratio). The reaction was performed with stirring at 60 °C under N_2 atmosphere. After the reaction, the particles were extracted with MeOH to remove un-bound linear pGMMA and then dried at 50 °C under vacuum. The resulting pGMMA modified MIP particles were denoted as H-MIP.

The H-MIP or MIP particles were dry-packed into stainless steel columns (50 × 4.6 mm). The recoveries of BSA from the columns were evaluated by HPLC with a Shimadzu LC-20A instrument. The BSA aqueous solution was injected and detected at 280 nm. The recoveries of BSA were determined by the comparison of the peak area of BSA eluted from the column with that obtained without column. The recoveries of humic acids from H-MIP or MIP column were also determined with the same method except that the detection wavelength was 230 nm.

2.7. Determination of sulfonylurea residue from soil by online SPE/HPLC

2.7.1. Preparation and extraction of spiked soil samples

Dry soil sample (10 g) was weighed and mixed with 10.0 mL of standard sulfonylurea solution (acetonitrile as the solvent). The mixture was sonicated for 45 min and then left standing for 12 h. The acetonitrile was then evaporated in a ventilation cabinet at the room temperature to obtain dry soil samples. The spiked samples were extracted with 30 mL of 0.2 mol L^{-1} ammonia in H_2O/MeOH (1:1, v/v). After being sonicated for 45 min and shaken for 1 h, the mixture was filtered under vacuum. The filtrate was dried at 60 °C in a rotary evaporator. The residues were dissolved in 2.0 mL acetonitrile for the SPE/HPLC analysis.

2.7.2. Online SPE/HPLC determination

A column-switching HPLC system (Fig. 2) was used for the determination of sulfonylurea residue in the soil extract. The system was composed of three LC-20 AT pumps, a SPD-20A UV detector (Shimadzu, Japan) and a 10-port switching valve (C2H-1000EH, VICI Valco instruments Co. Inc., USA). The position of the 10-port switching valve was controlled by the Vacom software (Valco instruments Co. Inc., USA). A Rheodyne 7725i injector with a 500 μL loop was used for the sample injection. H-MIP particles were dry-packed in a stainless steel column (50 × 4.6 mm) for the SPE sample pretreatment, which is coupled with an analytical column (150 × 4.6 mm, octadecyl Gemini, 5 μm, Phenomenex, USA). In the analysis, the H-MIP column was equilibrated with eluent A [(0.05 mol L^{-1} citric acid–0.1 mol L^{-1} Na_2HPO_4, pH 5.0)/MeCN, (9/1, v/v)] first. After the sample injection, the H-MIP column was washed with eluent A for 2 min at a flow rate of 1.0 mL min^{-1} (pretreatment step in Fig. 2, position A). Then the valve was switched to the position B. The sulfonylureas were eluted from the H-MIP column by a back-flush mode and analyzed on the analytical column using gradient elution composed by eluents B and C. The mobile phase B was (0.05 mol L^{-1} citric acid–0.1 mol L^{-1} Na_2HPO_4, pH 3.2)/MeCN, (6/4, v/v) and C was (0.05 mol L^{-1} citric acid–0.1 mol L^{-1} Na_2HPO_4, pH 4.0)/MeCN (4/6, v/v). The following gradient was used: 2–2.6 min, 0% B; 2.6–5.0 min, 90% B; 5–15 min, 90–81.6% B. The flow rate gradient was: 0–5 min, 1.0 mL min^{-1}; 5–15 min, 1.7 mL min^{-1}. The analytes were detected at 240 nm.

3. Results and discussion

To have an efficient sample extraction in the determination of sulfonylurea herbicide, we used RAFT polymerization to synthesize a bifunctional MIP. The synthesis scheme is presented in Fig. 3. Meanwhile, a new porogenic system was used in the precipitation polymerization. The object is to increase the surface area as well as the binding affinity of the MIP.

3.1. Selection of the synthetic conditions in the RAFT precipitation polymerization for the MIP preparations

In the first step of the material preparation, pyrazosulfuron-ethyl imprinted polymer was synthesized by RAFT precipitation polymerization (RAFTPP). The effect of RAFT chain transfer agent (CTA), porogens and rotation speeds on the properties of the MIP

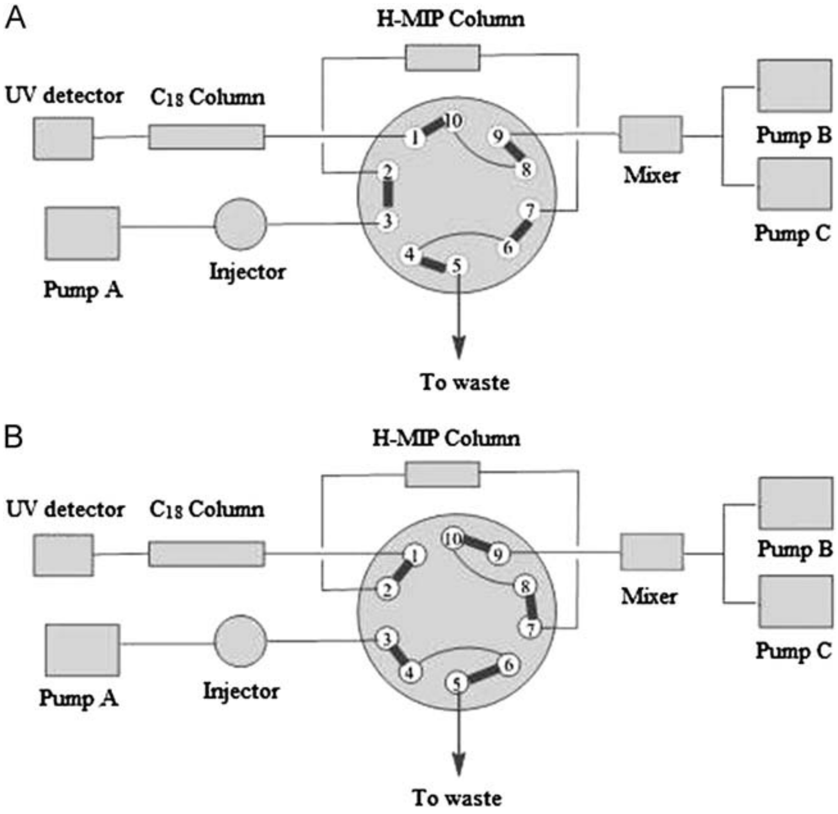


Fig. 2. The online SPE/HPLC system for the sulfonylurea herbicide analysis.

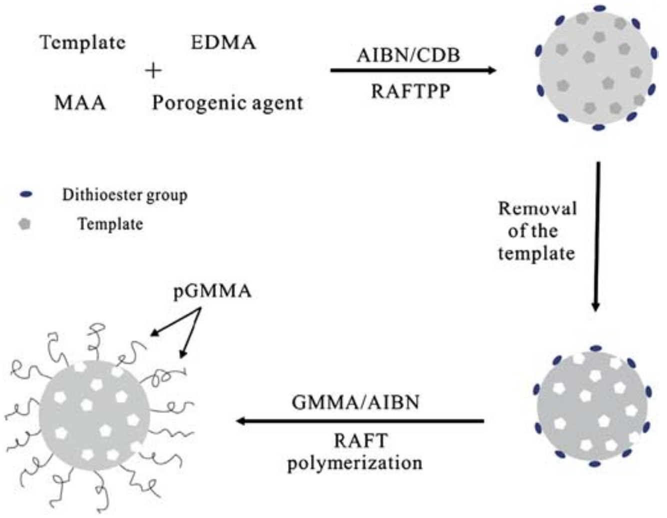


Fig. 3. Synthesis of bifunctional pyrazosulfuron-ethyl imprinted polymer with pGMMA layers by the RAFT polymerization.

Table 1
The conditions in the synthesis of pyrazosulfuron-ethyl imprinted polymers via RAFTPP[a].

Polymer	RAFT CTA	Porogenic agent	Ratio of PEG[b]	Rotation speed (rpm)
P1	DBTTC	MeCN	–	125
P2	CDB	MeCN	–	125
P3	CDB	MeCN/toluene (90/10, v/v)	–	125
P4	CDB	PEG-300/MeCN	1.6	125
P5	CDB	PEG-5000/MeCN	1.6	125
P6	CDB	PEG-12,000/MeCN	1.6	125
P7	CDB	PEG-300/MeCN	0.8	125
P8	CDB	PEG-300/MeCN	3.2	125
P9	CDB	PEG-300/MeCN	1.6	70
P10	CDB	PEG-300/MeCN	1.6	0
P9-NIP	CDB	PEG-300/MeCN	1.6	70

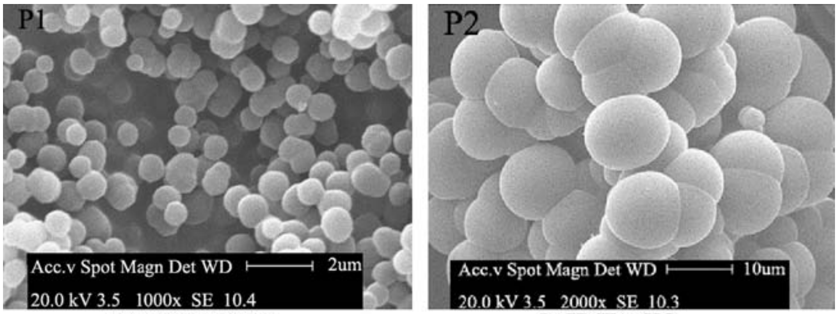
[a] The conditions which were the same in all experiments are listed in Section 2.2.

[b] The ratio of PEG was calculated by $m_{PEG}/(m_{MAA}+m_{EDMA})$, where m_{PEG}, m_{MAA}, and m_{EDMA} were the mass of PEG, MAA and EDMA, respectively.

was studied in order to optimize the synthesis conditions. The conditions investigated in the MIP preparations are listed in Table 1. Other reaction conditions were kept constant in the synthesis (Section 2.2).

3.1.1. Selection of RAFT chain transfer agent in the MIP synthesis

In the previous study [23], we used dibenzyltrithiocarbonate (DBTTC), a trithiocarbonate as chain transfer agent (CTA) in the pyrazosulfuron-ethyl imprinting by the RAFTPP method. We found that the resulting MIPs have small surface area (16 m^2/g) and low binding capacity for the template. In this study, cumyl dithiobenzoate (CDB), a bithiocarbonate) was used as the CTA in RAFTPP. For comparison, P1 and P2 were synthesized using CDB and DBTTC respectively while other conditions were kept the same in the preparations (Table 1). The MIP affinities for pyrazosulfuron-ethyl were evaluated with the equilibrium adsorption experiments (Fig. S1). The results indicated that the P2 synthesized with CDB has much higher binding capacity than P1 with DBTTC. Meanwhile, P2 has larger particle diameter revealed by the scanning electron microscopy (SEM) (Fig. 4). According to the general polymerization principle, fast chain propagation will result in early polymer precipitation which is attributed to reason of smaller particle size of P1 using DBTTC. While P2 has larger particle size



RAFT CTA: DBTTC

RAFT CTA: CDB

Fig. 4. SEM images of the MIPs synthesized with different RAFT reagents.

which may results from the slower polymerization rate by using CDB.

In the HPLC experiment, P2 not only has much lower column pressure (4.5 MPa for P2 versus 19.6 MPa for P1 when the mobile phase flow rate was 1.0 mL min^{-1}). The P2 also has stronger retention ($k=16.63$) for the template than P1 ($k=1.51$) in HPLC. These characters are favorable in the solid-phase extraction, therefore the CDB was selected as the CTA in the subsequent experiment.

3.1.2. *Influence of porogen and rotation speed on the properties of the MIPs*

3.1.2.1. The selection of porogen and rotation speed on the morphology of the MIPs. In the precipitation polymerization, the polymeric particles with low surface areas are often produced because the polymer grow by continual capturing of monomers and oligomers and the non-porous polymers are often produced by this mechanism. We used PEG as the porogen in this study, in order to create pores in the polymeric structures and to make polymers with higher surface areas. The PEGs with different molecular weight and PEG/monomer ratios were tested in the synthesis (Table 1). Acetonitrile/toluene was also used as the porogen for comparison. The SEM images of the MIPs synthesized using different porogenic reagents are shown in Fig. 5A. It has been found that MIP (P3) synthesized in acetonitrile/toluene has very small particle size, which resulted in high HPLC back pressure. Therefore, the acetonitrile/toluene is not the suitable porogen system for this study.

The MIPs: P4, P5 and P6 were synthesized using PEGs with different molecular weights for comparison. We found that microspheres with lower polydispersity were obtained with PEG-300/acetonitrile (P4), whereas PEG-5000/acetonitrile (for P5) and PEG-12,000/acetonitrile (for P6) resulted in coagulated particles (Fig. 5A). The experimental results also showed that PEG ratio in the synthesis influences the morphology of the polymers. When the PEG-300 ratio $[m_{\text{PEG}}/(m_{\text{MAA}}+m_{\text{EDMA}})]$ was lower or higher than 1.6 (for P7 and P8), the particle coagulation was more serious. While the P4 synthesized with the PEG ratio of 1.6 has better morphology. This porogenic condition was employed in the subsequent experiments.

In this MIP synthesis, reaction flasks were rotating at certain speed to provide a disturbance in the polymerization. Three kinds of rotation speeds were used in the polymerization (Table 1). The morphology of the resulting MIPs (P4, P9 and P10) was observed by scanning electron microscopy. The SEM images (P4 in Fig. 5A, P9 and P10 in Fig. 5B, respectively) have shown that the rotation speed of 70 rpm resulted in the best morphology (P9) in terms of particle dispersion and uniformity. High particle polydispersity was obtained when the polymerization was performed in a static solution (P10).

3.1.2.2. The effect of PEG porogen and rotation speed on the MIPs surface area and binding affinity. To study the relations between the synthetic conditions and MIP binding affinity, the binding isotherms of the MIPs synthesized using PEGs with different molecular weight and rotation speeds were obtained (Fig. 6). The Freundlich analysis was performed to obtain more information about the binding affinity. Meanwhile, the N_2 adsorption experiment was used to determine the surface areas of the MIPs (Table 2). In the low analyte concentration region of the binding isotherms, we found that MIPs synthesized under low or no rotation (for P9 and P10) have higher binding capacities than other MIPs. Because the high affinity binding sites in MIP are occupied first in the binding experiment, this phenomenon demonstrated that the less disturbance of the reaction solution is in favor of forming cavities with high affinity.

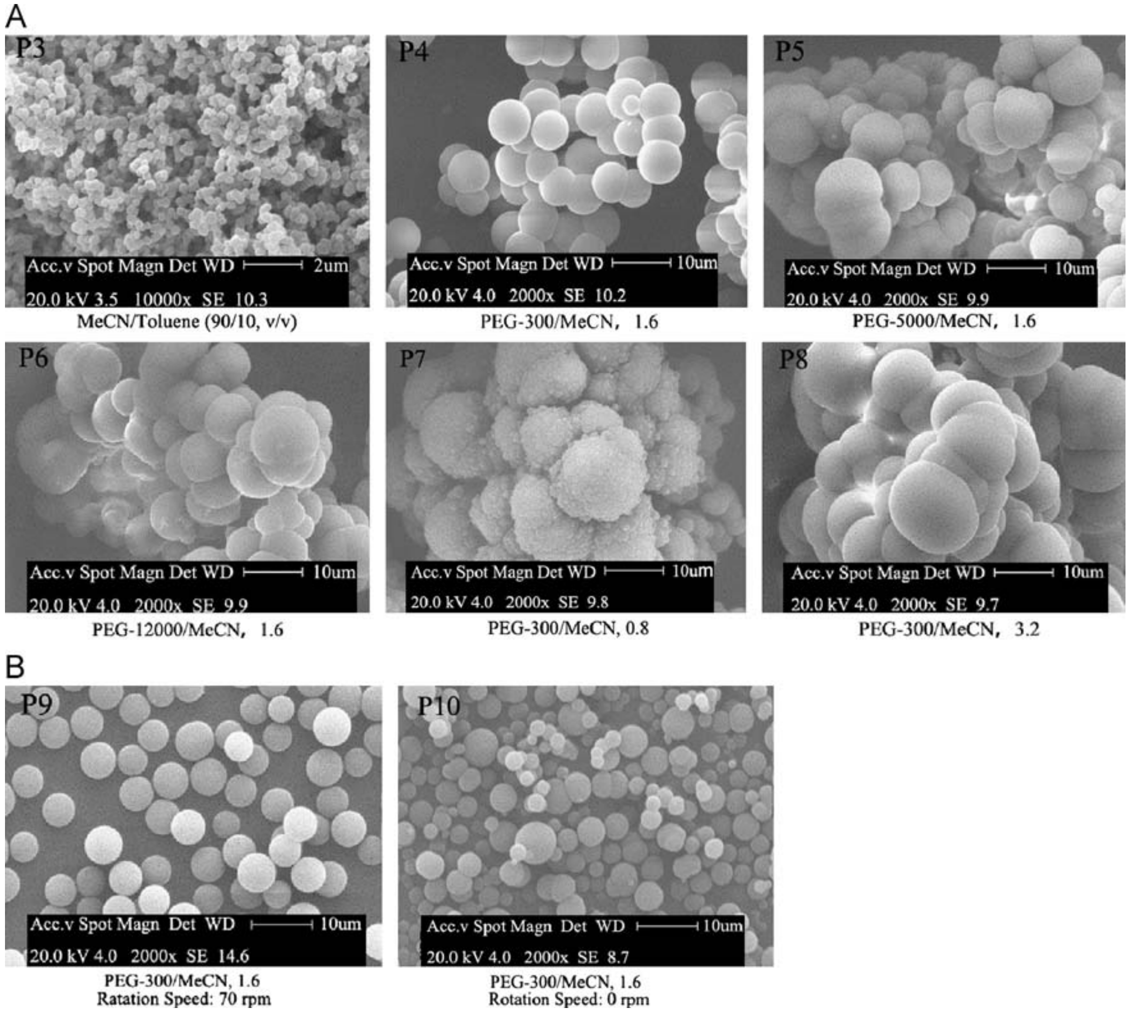
The Freundlich analysis results (Table 2) has shown that the MIPs synthesized with the same rotation speed (125 rpm) have binding constants in the range of 700–900 (for P2, P4, P5 and P6). While the P2 and P10 have highest binding capacities due to the existence of more binding sites in the polymers (Table 2). However, their surface areas cannot be measured by N_2 adsorption experiment. We assumed that the P2 and P10 are gel-like polymers in which the pores are opened-up in the binding solvent and collapsed in the dry state. By using PEG as the porogen in the polymerization, the MIPs with surface area more than 32 m^2/g were obtained. Meanwhile, the MIP surface area decreased as the molecular weight of the PEG increasing from 5000 (for P5) to 12,000 (for P6), which is attributed to the increase of the pores size in the polymer. It is demonstrated that PEG as the porogen enlarges the pores size and makes them big enough to be measured by the BET experiment. Since P9 has uniform particle size and good binding affinity, it was used in the subsequent studies.

3.2. *Selectivity of the pyrazosulfuron-ethyl imprinted polymer*

In order to evaluate the imprinting effects of the MIPs, the adsorption isotherm on P9 was compared with its corresponding non-imprinted polymer (P9-NIP). The adsorption capacity of the P9 is much higher than that of the P9-NIP (Fig. S2), indicating that the molecular imprinting has created specific affinity to the template molecules.

To have a further evaluation of the imprinting factor and selectivity of the imprinted polymer, the retentions of the pyrazosulfuron-ethyl and some sulfonylurea herbicides (see Fig. S-3 for their structures) on the MIP and NIP stationary phase were studied by the HPLC experiments. The P9 and P9-NIP particles were packed into stainless steel columns (50 × 4.6 mm) for evaluation. The retention factors and selectivity factors obtained from the experiment (Table 3).

The imprinting factors of P9 for the tested sulfonylureas are greater than 4, indicating that the MIP has better affinity for these compounds than P9-NIP. The P9 also has shown a good selectivity for other sulfonylurea compounds except bensulfuron-methyl. The stronger retention of the chlorimuron-ethyl on the P9 is due to the non-specific interaction, which can be seen from its higher retention on P9-NIP (Table 3).



MeCN/Toluene (90/10, v/v)

PEG-300/MeCN, 1.6

PEG-5000/MeCN, 1.6

PEG-12000/MeCN, 1.6

PEG-300/MeCN, 0.8

PEG-300/MeCN, 3.2

PEG-300/MeCN, 1.6
Ratation Speed: 70 rpm

PEG-300/MeCN, 1.6
Rotation Speed: 0 rpm

Fig. 5. SEM images of the MIPs synthesized using different porogents or rotation speeds. The porogenic reagent and ratio of PEG used in the corresponding polymerization are listed in the first line under the SEM photographs. (A) MIPs synthesized with different porogen (CDB as RAFT agent) and (B) MIPs synthesized with different rotation speed.

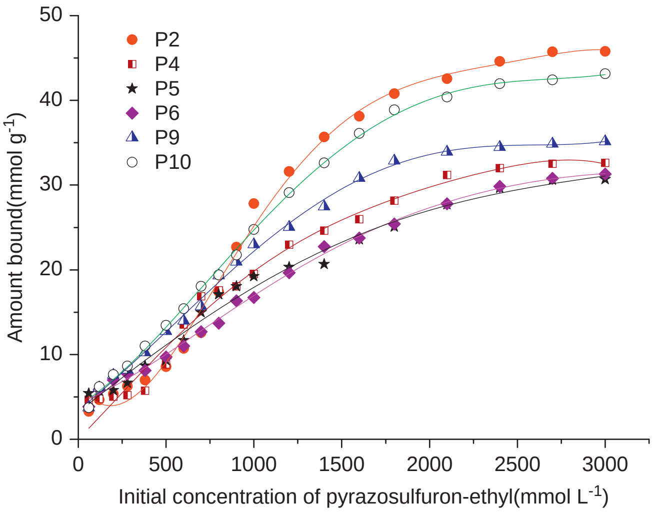


Fig. 6. Equilibrium adsorption isotherms of the MIPs for pyrazosulfuron-ethyl.

Table 2
The surface areas (BET) and the Freundlich analysis results from the MIPs synthesized with different conditions.

MIP	Surface area[a] (m^2/g)	Freundlich analysis results[b]				
		log a	*m*	*Determination coefficient R^2*	*N* (μmol g^{-1})	*K* ($L \cdot mol^{-1}$)
P2	–	0.083	0.476	0.941	23.38	768
P4	41.0	−0.149	0.497	0.962	15.69	898
P5	43.7	0.014	0.434	0.973	12.05	777
P6	32.2	−0.611	0.627	0.970	13.83	846
P9	68.3	−0.367	0.585	0.940	17.51	1046
P10	–	−0.383	0.611	0.932	20.66	909

[a] The surface area of P2 and P10 cannot be measured by the N_2 adsorption experiment.

[b] The data from initial PS concentration above 500 mmol L^{-1} were used in the Freundlich analysis.

3.3. *Surface grafting of pGMMA chains on the MIP microspheres*

In the restricted access materials, physical or chemical diffusion barriers are used for macromolecular exclusion. Meanwhile, the diffusion barriers are hydrophilic to prevent protein precipitation and irreversible adsorption. In this study, pGMMA were grafted on the surface of the P9 via RAFT polymerization to form hydrophilic polymer external layers, which prevent proteins and humic acids from being adsorbed on the MIP core structures.

In the RAFT grafting reaction, the dithioester groups bound in the MIP were employed as the chain transfer agent (Fig. 7). The

Table 3
The imprinting and selective factors of the MIP.

Sulfonylurea herbicides	Retention factor(k)		Imprinting factor k_{MIP}/k_{NIP}	Selective factor (α)
	P9	P9-NIP		P9
Pyrazosulfuron-ethyl	13.7	2.9	4.7	–
Bensulfuron-methyl	12.4	2.7	4.6	1.1
Chlorimuron-ethyl	26.4	6.2	4.3	–
Monosulfuron	6.0	1.4	4.5	2.3
Chlorosulfuron	7.0	1.6	4.3	1.9

The mobile phase was aqueous solution (pH 2.5, adjusted by phosphoric acid)/MeCN, (6/4, v/v). The flow rate was 1.0 mL min^{-1}. The selectivity factors were calculated by k_P/k_X, where k_P and k_X are the retention factors of pyrazosulfuron-ethyl and other sulfonylurea herbicides, respectively.

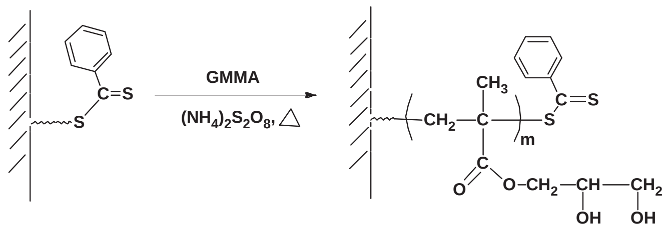


Fig. 7. Grafting of pGMMA chains on the surface of the imprinted polymer via the RAFT polymerization.

$(NH_4)_2S_2O_8$ was used as the radical initiator. In the study, the GMMA concentration and the polymerization time were optimized to create proper pGMMA chain length. The ideal hydrophilic layers should have suitable thickness which has enough coverage on the inner core and do not have non-ideal effects on the template retention.

The relation between the reaction conditions and macromolecule exclusion ability was evaluated by the recoveries of BSA from columns packed with pGMMA grafted MIP materials (Table 4). Almost complete BSA recovery was achieved from the H-MIP-2 stationary phase. Whereas low BSA recovery (50.1%) was observed when the P9 was used. This result demonstrated that pGMMA chain provided the barrier for protein diffusion.

The recovery of the humic acids on the H-MIP-2 was also analyzed using (0.05 mol/L citric acid–0.1 mol/L Na_2HPO_4, pH 5.0)/MeCN, (9/1, v/v) as the mobile phase. The recovery of humic acids on the H-MIP-2 column is 89.4%, which is much higher than the recovery (30.3%) on the P9 column. Meanwhile, template molecule (pyrazosulfuron-ethyl) retained strongly and could not be eluted within 60 min on the H-MIP-2 column.

3.4. Determination of sulfonylurea residue from soil by the SPE/HPLC online coupling method

3.4.1. Study of the influence of mobile phase on the retention of the analyte on the H-MIP

A method for the determination of residues of three sulfonylurea compounds was developed by online SPE/HPLC using H-MIP-2 as the SPE material. The influence of mobile phase on the retentions of the analyte on the H-MIP column was studied first. The relation between the mobile phase pH and retention of PS was investigated (Fig. 8A). The buffers pH varied from 4.0 to 7.0 was prepared by 0.1 mol L^{-1}citric acid–0.2 mol L^{-1} Na_2HPO_4. The result has shown that the retention of the PS decreased with the increase of pH value. This phenomenon was attributed to the more ionization of PS in the higher pH environment, which resulted in less H-bonding and non-specific hydrophobic interaction between the PS and H-MIP. The result also shows that the retention difference between these two materials is small although the retention of PS on the H-MIP-2 is weaker than that on the P9.

Table 4
The BSA recoveries from the MIP and pGMMA grafted MIPs synthesized under different reaction times in the grafting polymerization.

Materials[a]	H-MIP-1	H-MIP-2	H-MIP-3	P9
Concentration of GMMA (mol L^{-1})	0.165	0.330	0.330	–
Reaction time (h)	6	6	4	–
Recovery of BSA (%)[b]	88.9	98.8	92.0	50.1

[a] The H-MIP-1, H-MIP-2 and H-MIP-3 are the materials after pGMMA modification of the P9.

[b] HPLC column size was 50 × 4.6 mm. The mobile phase was (0.1 M phosphate buffer, pH 7.0)/THF/isopropanol (84/6/10, v/v). The concentration of BSA was 5 mg mL^{-1}. Injection volume was 20 μL. BSA was detected at 280 nm.

The effect of the ratio of buffer/MeOH on the retention of PS was also studied (Fig. 8B). The mobile phases: (0.1 mol L^{-1}citric acid–0.2 mol L^{-1} Na_2HPO_4, pH 5.0)/MeOH with different buffer/MeOH ratios were employed in the analysis. We assumed that in the pH 5.0 mobile phase, the PS is in a non-ionization status. The PS retention on the H-MIP increased with the increase of the buffer ratio in the mobile phase, which showed a reversed-phase mechanism. When the percentage of buffer was more than 50, the retention of the PS on the P9 was higher than that on the H-MIP, which demonstrated that the H-MIP is more hydrophilic.

3.4.2. Validation of the SPE/HPLC online coupling method

After the analytical conditions optimization, the SPE/HPLC method for the determination of three sulfonylurea herbicides in soil was established. Spiked soil samples were used for the method validation. The PS standard solutions were used in the HPLC calibration curve establishment. A linear calibration curve was obtained in the analyte concentrations range of 100–5000 ng mL^{-1} with determination coefficient greater than 0.999.

The accuracy of the method was determined through the recovery tests. The fortified soil sample extracts with three spiked concentration levels were analyzed. The results are presented in Table 5. The recoveries from 81% to 99% with the RSD less than 6.6% were obtained. The limit of detection (LOD) and limit of quantification (LOQ) are < 4.8 μg kg^{-1} and < 15.9 μg kg^{-1}, defined as three times and 10 times of the noise of HPLC profile respectively (Table 5).

The chromatogram of the sulfonylurea residue in spiked soil sample from SPE/HPLC is displayed in Fig. 9. It can be seen that three analytes are well separated and a clean base-line was obtained after the online SPE process.

4. Conclusions

A pyrazosulfuron-ethyl imprinted polymer with pGMMA external layers was synthesized by the reversible addition–fragmentation chain transfer polymerization. PEG has been used as the polymeric porogen, which increased the surface areas of the MIPs. It is a new method in making porous materials in the precipitation polymerization. The pGMMA on the MIP microsphere forms a hydrophilic shell which plays a role of humic acids exclusion. The result demonstrated that the RAFT polymerization is a facile method for the preparation of core-shell material with both properties of MIP and RAM. This material can be used for the efficient sample extraction and clean-up in the sulfonylurea residue analysis from environmental samples.

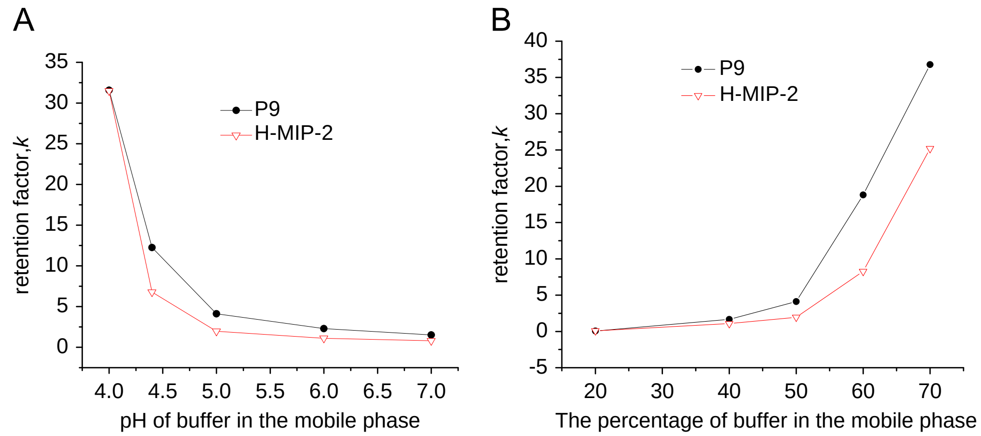


Fig. 8. The influence of the mobile phase on the retention of PS. The column was a stainless steel column (50 × 4.6 mm) packed with H-MIP-2. The analyte was detected by 240 nm. The flow rate was 1.0 mL min^{-1}. In (A), the mobile phase was (0.1 mol L^{-1}citric acid–0.2 mol L^{-1} Na_2HPO_4)/MeOH, (50/50, v/v). In (B), the mobile phase was (0.1 mol L^{-1}citric acid–0.2 mol L^{-1} Na_2HPO_4, pH 5.0)/MeOH.

Table 5
The figures of merit of the column-switching HPLC method for the determination of three sulfonylurea herbicides ($n=3$).

Compounds	Spiked (μg kg^{-1})	Found (μg kg^{-1})	Recovery (%)	RSD (%)	LOD (μg kg^{-1})	LOQ (μg kg^{-1})
BSM	40	33.8	84.4	1.8	4.1	13.7
	100	95.6	95.6	1.1		
	200	198.2	99.1	0.9		
PS	40	33.9	84.7	4.7	4.6	15.3
	100	94.2	94.2	2.8		
	200	197.6	98.8	0.9		
CME	40	32.8	81.9	6.6	4.8	15.9
	100	89.5	89.5	1.3		
	200	191.6	95.8	5.8		

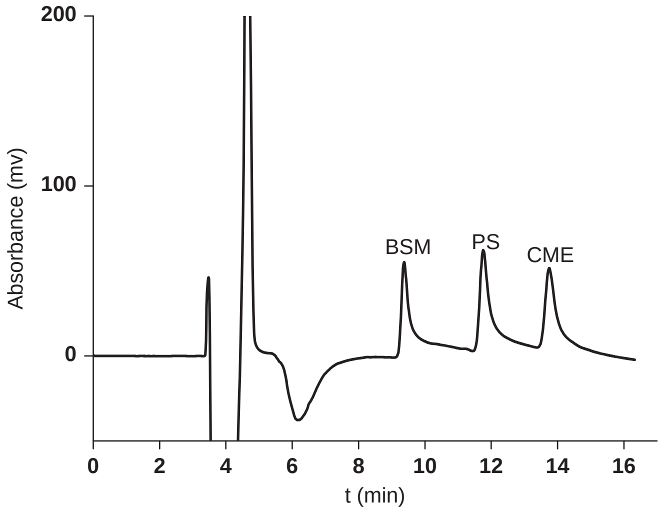


Fig. 9. Chromatogram of spiked soil extracts by online-SPE/HPLC. The SPE column was 50 × 4.6 mm packed with H-MIP. The analytical column: octadecyl Gemini, 5 μm (150 × 4.6 mm). The elution conditions are listed in Section 2.7. The injection volume was 500 μL. The analytes were detected at 240 nm.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2013.03.078.

References

[1] P.S. Sharma, F. D'Souza, W. Kutner, Trends Anal. Chem. 34 (2012) 59–77.
[2] G. Vasapollo, R.D. Sole, L. Mergola, M.R. Lazzoi, A. Scardino, S. Scorrano, G. Mele, Int. J. Mol. Sci. 12 (2011) 5908–5945.
[3] J. Haginaka, J. Sep. Sci. 32 (2009) 1548–1565.
[4] A. Pardo, L. Mespouille, P. Dubois, P. Duez, B. Blankert, Cent. Eur. J. Chem. 10 (2012) 751–765.
[5] A. Beltran, F. Borrull, R.M. Marcé, P.A.G. Cormack, Trends Anal. Chem. 29 (2010) 1363–1375.
[6] A. Abouzarzadeh, M. Forouzani, M. Jahanshahi, N. Bahramifar, J. Mol. Recognit. 25 (2012) 404–413.
[7] J. Haginaka, C. Miura, N. Funaya, H. Matsunaga, Anal. Sci. 28 (2012) 315–317.
[8] Y. Shi, H. Lv, X. Lu, Y. Huang, Y. Zhang, W. Xue, J. Mater. Chem. 22 (2012) 3889–3898.
[9] L. Ye, P.A.G. Cormack, K. Mosbach, Anal. Chim. Acta 435 (2001) 187–196.
[10] S. Polati, M. Bottaro, P. Frascarolo, F. Gosetti, V. Gianotti, M. Gennaro, Anal. Chim. Acta 579 (2006) 146–151.
[11] Z. Chen, R. Kookana, R. Naidu, Chromatographia 52 (2000) 142–146.
[12] J. Rouchaud, O. Neus, C. Moulard, Int. J. Environ. Anal. Chem. 79 (2001) 65–80.
[13] Q.Z. Zhu, P. Degelmann, R. Niessner, D. Knopp, Environ. Sci. Technol. 36 (2002) 5411–5420.
[14] J. Bastide, J.P. Cambon, F. Breton, S.A. Piletsky, R. Rouillon, Anal. Chim. Acta 542 (2005) 97–103.
[15] X.C. Dong, N. Wang, S.L. Wang, X.W. Zhang, Z.J. Fan, J. Chromatogr. A 1057 (2004) 13–19.
[16] Y. Peng, Y. Xie, J. Luo, L. Nie, Y. Chen, L. Chen, S. Du, Z. Zhang, Anal. Chim. Acta 674 (2010) 190–200.
[17] X. Liu, Z. Chen, R. Zhao, D. Shangguan, G. Liu, Y. Chen, Talanta 71 (2007) 1205–1210.
[18] L. Guo, Q. Deng, G. Fang, W. Gao, W. Shuo, J. Chromatogr. A 1218 (2011) 6271–6277.
[19] E.A. Hogendoorn, E. Dijkman, B. Baumann, C. Hidalgo, J.V. Sancho, F. Hernandez, Anal. Chem. 71 (1999) 1111–1118.
[20] H.Takehira Haginaka, K. Hosoya, N. Tanaka, J. Chromatogr. A 849 (1999) 331–339.
[21] F. Puoci, F. Iemma, G. Cirillo, M. Curcio, O.I. Parisi, U.G. Spizzirri, N. Picci, Eur. Polym. J. 45 (2009) 1634–1640.
[22] W. Xu, S. Su, P. Jiang, H. Wang, X. Dong, M. Zhang, J. Chromatogr. A 1217 (2010) 7198–7207.
[23] L. Yang, Z. Fan, T. Wang, W. Cai, M. Yang, P. Jiang, M. Zhang, X. Dong, Anal. Lett. 44 (2011) 2617–2632.
[24] H. Sambe, K. Hoshina, J. Haginaka, J. Chromatogr. A 1152 (2007) 130–137.
[25] Y. Watabe, K. Hosoya, N. Tanaka, T. Kubo, T. Kondo, M. Morita, J. Chromatogr. A 1073 (2005) 363–370.

[26] S. Xu, J. Li, L. Chen, Talanta 85 (2011) 282–289.
[27] G. Pan, B. Zu, X. Guo, Y. Zhang, C. Li, H. Zhang, Polymer 50 (2009) 2819–2825.
[28] M. Turson, X.L. Zhuang, H.N. Liu, P. Jiang, X.C. Dong, Chin. Chem. Lett. 20 (2009) 1136–1140.
[29] H. Liu, X. Zhuang, M. Turson, M. Zhang, X. Dong, J. Sep. Sci. 31 (2008) 1694–1701.
[30] M.R. Halhalli, C.S.A. Aureliano, E. Schillinger, C. Sulitzky, M.M. Titirici, B. Sellergren, Polym. Chem. 3 (2012) 1033–1042.
[31] C. Gonzato, M. Courty, P. Pasetto, K. Haupt, Adv. Funct. Mater. 21 (2011) 3947–3953.
[32] S. Xu, J. Li, L. Chen, J. Mater. Chem. 21 (2011) 4346–4351.
[33] A.W.M. Lee, W. Chan, H. Wong, Synth. Commun 18 (1988) 1531–1536.
[34] X.Q. Xin, Y.M. Wang, C.Y. Pan, Acta Polym. Sin. 3 (2005) 398–402.
[35] A.M. Rampey, R.J. Umpleby, G.T. Rushton, J.C. Iseman, R.N. Shah, K.D. Shimizu, Anal. Chem. 76 (2004) 1123–1133.