

# Preparation and Characterization of Uniform Molecularly Imprinted Polymer Beads for Separation of Triazine Herbicides

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**ABSTRACT:** Uniform molecularly imprinted polymer beads were synthesized by precipitation polymerization for separation of triazine herbicides. A series of imprinted polymers were prepared using ametryn as template and divinylbenzene as crosslinking monomer, in combination with three different functional monomers under different solvent conditions. Under optimized reaction conditions, we obtained uniform molecularly imprinted polymer microspheres that display favorable molecular binding selectivity for triazine herbicides. The imprinted polymer beads synthesized using methacrylic acid as functional monomer in a mixture of methyl ethyl ketone and heptane showed the best results in

terms of particle size distribution and molecular selectivity. Compared with nonimprinted polymer microspheres, the imprinted microspheres displayed significantly higher binding for a group of triazine herbicides including atrazine, simazine, propazine, ametryn, prometryn, and terbutryn. For the first time, precipitation polymerization has been used to produce highly uniform imprinted microspheres suitable for affinity separation of triazine herbicides. © 2012 Wiley Periodicals, Inc. *J Appl Polym Sci* 000: 000–000, 2012

**Key words:** molecular imprinting; precipitation polymerization; triazine; ametryn; microspheres

## INTRODUCTION

Molecularly imprinted polymers (MIPs) are synthetic materials containing molecular binding sites with template-defined size, shape, and functional groups. The molecular binding sites are formed using specific template-functional monomer interaction to hold the template in place during free radical polymerization. Because of their high stability and predictable molecular recognition specificity, MIPs have been used successfully in the fields of affinity separation,<sup>1–3</sup> chemical sensing,<sup>4–8</sup> catalysis,<sup>9</sup> and controlled drug delivery.<sup>10–12</sup>

Commonly used methods for MIP preparation include bulk polymerization,<sup>13</sup> suspension polymerization,<sup>14</sup> emulsion polymerization,<sup>15</sup> multistep swelling polymerization,<sup>16</sup> and precipitation polymeri-

zation.<sup>17</sup> Because of its simplicity, precipitation polymerization has become very attractive for preparation of MIP nanoparticles and microspheres with diameter in the range of from 100 nm to 10 μm.<sup>18–25</sup> In most of the studies performed on molecular imprinting in precipitation polymerization systems, methacrylic acid (MAA) has been used as the functional monomer, and the polymerization is often carried out in acetonitrile, which is a near theta solvent for polymethacrylate type of polymers. There are only limited investigations on the suitability of new functional monomers and reaction solvents for preparation of MIP beads under precipitation polymerization condition.<sup>26</sup>

Ametryn (2-ethylamine-4-(isopropylamine)-6-(methylthio)-s-triazine) is one of the triazine type chemicals that can inhibit photosynthesis and other enzymatic processes, and is used frequently as herbicides in agriculture. Ametryn can be absorbed through plant roots, and it can accumulate in the apical meristems.<sup>27</sup> The half-lives of triazine herbicides vary from weeks to several months and under environmental conditions are usually degraded to compounds that are more soluble in water. These herbicides and their degradation products can be retained by the organic matter in soil, resulting in the accumulation of these pollutants in the environment.<sup>28</sup> From the health and environmental point of view, triazine herbicides in the environment need to be closely monitored. To

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TABLE I  
Preparation of Ametryn-Imprinted Polymers

Polymer	Template (mmol)	Functional monomer (mmol)	DVB (mmol)	Particle size ( $\mu\text{m}$ )	Yield (%)	Reaction temp ( $^{\circ}\text{C}$ )	Solvent (mL)
MIP1	0.53	MAA (1.31)	5.25	1.6-2.5	35	60	MEK:heptane, 30:70 (40)
MIP2	0.53	Maleimide (0.53)	0.53	1.0	25	70	MEK:heptane, 70:30 (25)
MIP3	0.53	4VPy (1.31)	5.25	1.0	35	70	Acetonitrile (40)

achieve low detection limit for monitoring triazine herbicides, MIPs have been considered as highly useful adsorbents to realize effective sample enrichment and clarification, i.e., through solid phase extraction (SPE).<sup>24</sup>

The aim of this work was to investigate preparation of ametryn-imprinted polymer microspheres using new functional monomer and solvent under precipitation polymerization condition. Three different functional monomers (methacrylic acid, maleimide and 4-vinylpyridine) were used, in combination with divinylbenzene cross-linker, to prepare ametryn-imprinted polymer beads in a mixture of methylethyl ketone (MEK), and heptane. The main objective was to obtain uniform imprinted polymer beads with a general molecular selectivity for triazine herbicides.

## EXPERIMENTAL

### Reagents

Ametryn (98.8%), atrazine (99.2%), terbutryn (99.0%), divinylbenzene (DVB-55, technical grade, 55%, mixture of isomers), maleimide (99.0%), heptane (anhydrous 99.0%), methylethyl ketone (MEK), 4-vinylpyridine (4VPy, 95.0%) and 2,2'-azobisisobutyronitrile (AIBN, 98%) were purchased from Sigma-Aldrich (St. Louis, MO). DVB-55 was passed through an aluminum oxide column to remove the inhibitor *p*-tert-butylcatechol before use. Prometryn (98.8%), propazine (99.3%), simazine (99.0%), and acetonitrile (HPLC grade) were purchased from Fluka. Acetic acid (glacial, 100%) was obtained from Merck (Darmstadt, Germany). Methacrylic acid (MAA, 98.5%) was purchased from ACROS (Geel, Belgium). Solvents like MEK, heptane, and acetonitrile were used as received. Ultrapure deionized water was used for HPLC analysis. Stock solutions ( $1000 \mu\text{g mL}^{-1}$ ) of the triazine herbicides were prepared by dissolving 0.01 g of the herbicides in 10 mL acetonitrile. Standard solution ( $10\text{--}100 \mu\text{g mL}^{-1}$ ) for calibration was prepared by dilution of the stock solution with acetonitrile.

### Apparatus

Scanning electron microscopy (SEM) imaging was done on a JEOL JSM-6700F Field Emission Scanning Electron Microscope (Tokyo, Japan). Polymer micro-

spheres were sputter coated with gold prior to the SEM measurement. A Lachrom Merck Hitachi HPLC equipped with a L-7100 gradient pump, a L-7455 diode array detector, a L-7612 solvent degasser and a L-7200 auto sampler was used for quantification of the herbicides. All HPLC analysis was carried out on a Lichrospher RP-18 column (particle size  $5 \mu\text{m}$ , from Merck, Darmstadt Germany). A STOVALL hybridization oven (Greensboro, NC) was used to provide gentle rotation for the polymerization mixture, and a Branso 3200 (USA) ultrasonic cleaner was used to assist dispersion of polymer particles in solvent. Centrifugation was carried out with a Bio-fuge Pico centrifuge (Kendro Laboratory, Germany).

### HPLC conditions

Sample injection volume was  $10 \mu\text{L}$  and the column was maintained at room temperature of about  $25^{\circ}\text{C}$ . The mobile phase used was acetonitrile : water (70: 30, v/v, isocratic elution) at a flow rate of  $1.0 \text{ mL min}^{-1}$ . The UV absorbance was measured at  $254 \text{ nm}$ .

### Polymer synthesis

Precipitation polymerization was used for the synthesis of molecularly imprinted microspheres under the conditions given in Table I. Ametryn (acting as the template molecule) was dissolved in 40 mL of solvent in a  $150 \times 25 \text{ mm}$  borosilicate glass tube equipped with a screw cap. The functional monomer (MAA, maleimide, or 4VPy), the cross-linking monomer (DVB) and the initiator (AIBN) were then added. The solution was purged with a gentle flow of nitrogen for 5 min and then sealed. The borosilicate glass tube was fixed in a horizontal position in the hybridization oven, and was rotated at a speed of 20 rpm. Temperature was increased from 20 to  $60^{\circ}\text{C}$  or  $70^{\circ}\text{C}$  in 20 min, and then kept constant for 24 h. After polymerization, the solid particles were collected by centrifugation. The template was removed from MIP particles by batch mode solvent extraction using methanol containing 10% acetic acid (v/v), until no ametryn could be detected in the washing solvent using a UV-Vis spectrometer. The microspheres free of template were then washed with methanol and acetone, and then dried in a vacuum chamber. For comparison, nonimprinted

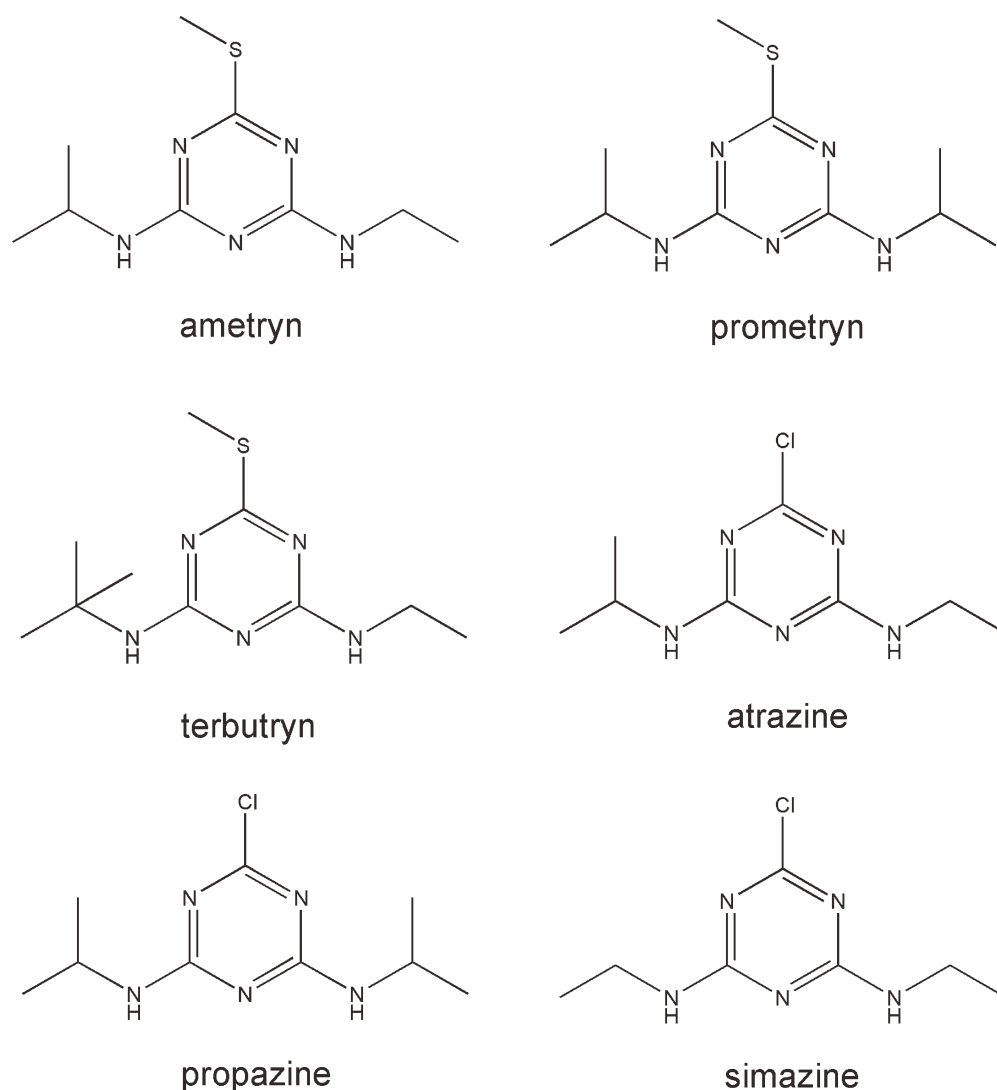


Figure 1 Chemical structure of the triazine herbicides.

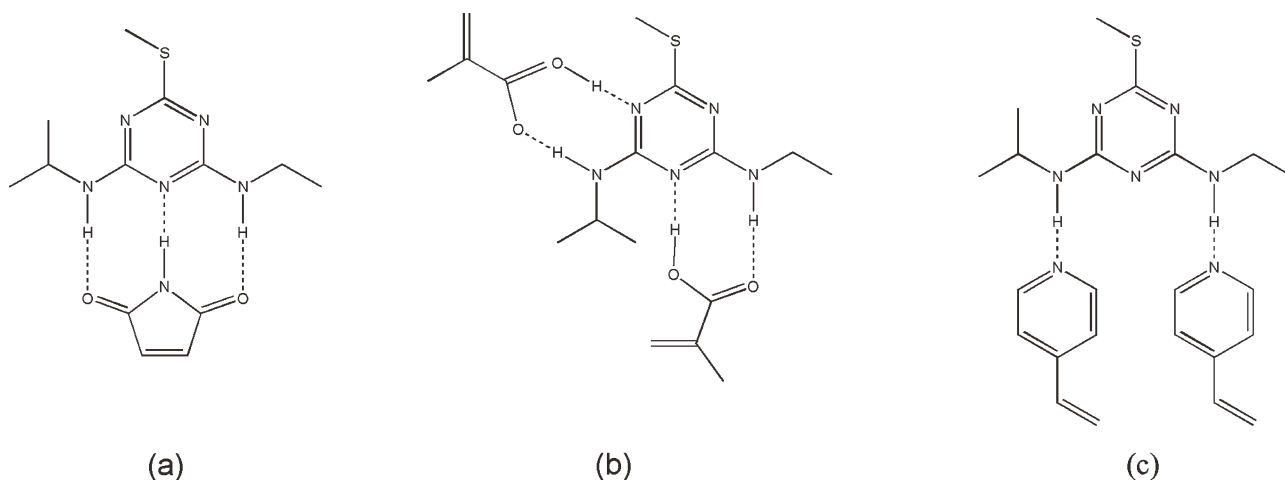
reference polymers were prepared under the same condition except that no template was added before the polymerization.

#### Equilibrium binding experiment

Polymer microspheres (10 mg) were taken into a 2.5-mL microcentrifuge tube containing 1 mL of herbicide solution (10  $\mu\text{g mL}^{-1}$  in acetonitrile). These microcentrifuge tubes were then incubated at room temperature on a rocking table for 24 h. After the incubation, samples were centrifuged at 15,000 rpm for 10 min to settle the particles. Supernatant was taken from each sample, passed through a 13 mm PTFE syringe filter (with pore size 0.2  $\mu\text{m}$ ), and was analyzed by HPLC. The amount of analyte bound to the polymer was calculated by subtraction of free fraction from the total amount added. The data obtained was mean value of triplicate independent samples.

#### RESULTS AND DISCUSSION

In a previous study, we used propranolol as template to synthesize molecularly imprinted polymer beads with controllable particle size in the range of 130 nm–2.4  $\mu\text{m}$ . Controlling of particle size was achieved by adjusting the ratio between two types of cross-linking monomers, DVB, and trimethylolpropane trimethacrylate (TRIM). The imprinted polymers synthesized in acetonitrile using MAA as functional monomer had very narrow size distribution, and displayed high molecular recognition specificity.<sup>29</sup> Considering SPE applications, in this work we selected to use DVB as cross-linker for preparation of ametryn-imprinted polymer beads. Three different functional monomers, MAA, maleimide, and 4VPy were used to investigate their possibility of forming high fidelity imprinted sites for triazine recognition. In addition to acetonitrile, a series of MEK : heptane mixture was tested as reaction



**Figure 2** Proposed hydrogen bond interactions between ametryn and maleimide (a), ametryn and MAA (b), and ametryn and 4VPy (c).

solvent in order to obtain uniform imprinted polymer beads. In previous studies, Stöver and coworkers suggested that when DVB was used as cross-linker, a solvent composition of MEK : heptane led to a transition from solution polymerization to precipitation polymerization.<sup>30</sup> Because the polarity of MEK : heptane is lower than acetonitrile, a stronger interaction between ametryn and the three functional monomers were expected, which should lead to a better molecular imprinting effect. Because of the structural similarity of all the triazine herbicides (Fig. 1), we expected that MIP beads prepared using ametryn template should display group selectivity, which is favorable for SPE application for simultaneous extraction of a group of compounds with similar structures.

#### Design of synthetic conditions for preparation of ametryn-imprinted polymers

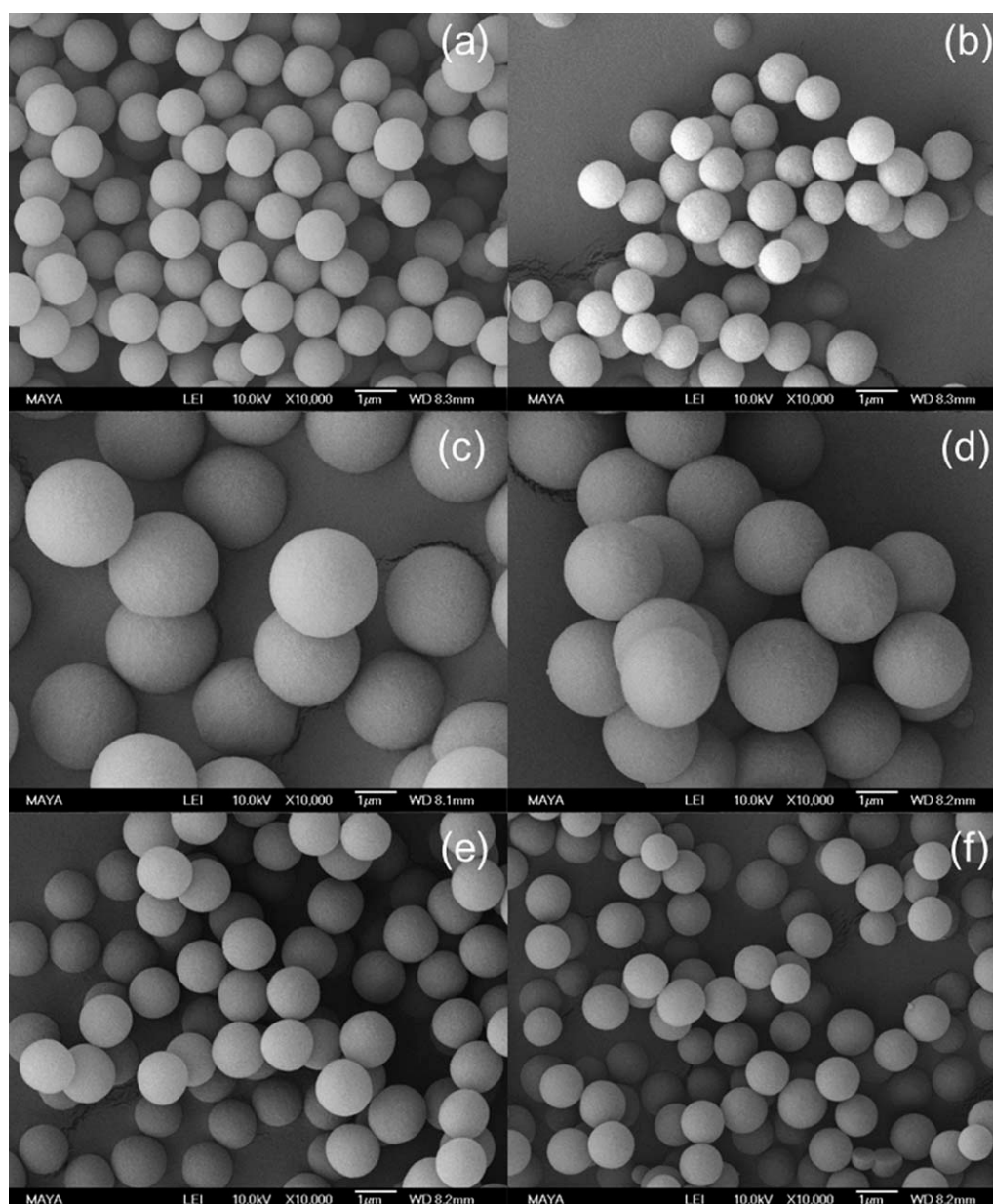
For preparation of ametryn-imprinted polymers, we investigated three different functional monomers,

MAA, maleimide and 4VPy, based on their possible modes of interaction with the template (Fig. 2). MAA has the possibility to form multiple hydrogen bonds with ametryn, and has been used in the past to synthesize ametryn-imprinted polymers using traditional bulk polymerization<sup>31–34</sup> and multistep swelling polymerization.<sup>35</sup> Maleimide is known to form tridentate hydrogen bonds with triazine-like structure after being incorporated into maleimide-styrene copolymer,<sup>36</sup> but this property has not been exploited for preparing molecularly imprinted polymers. The functional monomer 4VPy was considered as possible hydrogen bond acceptor to bind the ametryn template (Fig. 2).

The choice of using DVB as cross-linker was based on two reasons: one is that the size of polyDVB particles prepared by precipitation polymerization in acetonitrile or MEK: heptane mixture is in general larger than 1  $\mu\text{m}$ <sup>26,29</sup> and more suitable to pack SPE columns, the other consideration is that DVB may

**TABLE II**  
Effect of Reaction Solvent on Polymer Morphology

Polymer system	Reaction solvent	Observations
Poly(MAA-co-DVB)	MEK: heptane (20: 80)	Highly polydispersed, coagulum
	MEK: heptane (22: 78)	Highly polydispersed, coagulum
	MEK: heptane (25: 75)	Slightly polydispersed
	MEK: heptane (30: 70)	Monodispersed particles
	MEK: heptane (35: 65)	No polymer particle
	Acetonitrile	Slightly polydispersed particles
Poly(maleimide-co-DVB)	MEK: heptane (40: 60)	Gel
	MEK: heptane (50: 50)	Gel
	MEK: heptane (60: 40)	Gel
	MEK: heptane (70: 30)	Monodispersed particles
	MEK: heptane (80: 20)	Monodispersed particles
	MEK: heptane (90: 10)	Monodispersed particles
	MEK	Monodispersed particles
	Acetonitrile	Monodispersed particles
Poly(4VPy-co-DVB)	Acetonitrile	Monodispersed particles



**Figure 3** Scanning electron micrographs of MIP1 (a), NIP1 (b), MIP2 (c), NIP2 (d), MIP3 (e), and NIP3 (f).

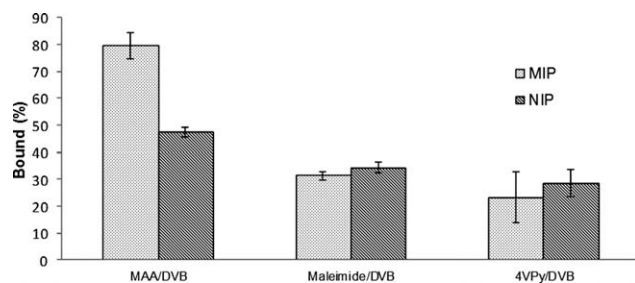
provide additional  $\pi$ - $\pi$  interaction with the template, thereby increase the molecular imprinting efficiency.<sup>37</sup>

The basic reaction conditions used to prepare ametryn-imprinted polymers are given in Table I. For MIP1 and MIP3, the molar ratio between the functional monomer and DVB-55 was kept as 1 : 4. This monomer feed translates into a nominal cross-linking density of  $\sim 69\%$ , which is common for preparation of molecularly imprinted polymers. For MIP2, equal moles of maleimide and DVB-55 were used to make sure that the cross-linked polymer have more homogeneous structure, i.e., the main chain of the polymer have alternating maleimide and styrene sequence<sup>36</sup> and be free of excess homopolymer segments.

#### Effect of reaction solvent on polymer morphology

The effect of reaction solvent on polymer morphology was first studied for the poly(MAA-*co*-DVB) system. A series of poly(MAA-*co*-DVB) was prepared in neat acetonitrile and MEK : heptane mixture, and the appearance of the polymer product was inspected visually or using an optical microscope. As described in Table II, when the volume ratio of MEK : heptane was 30 : 70, monodispersed poly(MAA-*co*-DVB) particles were obtained [Fig. 3(a,b)]. Use of neat acetonitrile as reaction solvent led to poly(MAA-*co*-DVB) microspheres with a slightly higher size distribution.

For the poly(maleimide-*co*-DVB) system, we found that gel formation took place at low MEK : heptane



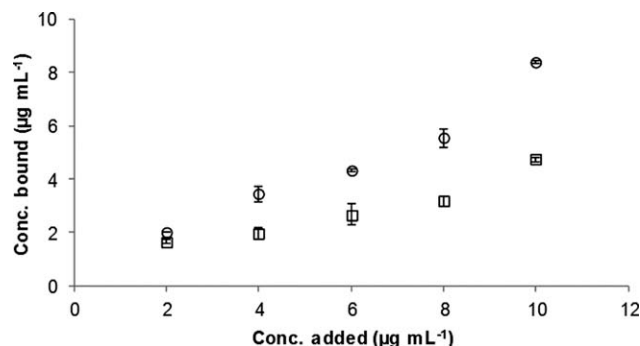
**Figure 4** Binding of ametryn by different polymers. Solvent: acetonitrile. Polymer conc.:  $10 \text{ mg mL}^{-1}$ . Initial conc. of ametryn:  $10 \text{ } \mu\text{g mL}^{-1}$ .

ratio (Table II). We also noticed that maleimide has a low solubility in heptane. By increasing the ratio of MEK : heptane from 60 : 40 to 70 : 30, we obtained monodispersed poly(maleimide-*co*-DVB) particles [Fig. 3(c,d)]. Further increase of MEK did not affect the uniformity of the polymer beads, however it resulted in decreased polymer yield. Using neat acetonitrile as reaction solvent gave monodispersed poly(maleimide-*co*-DVB) microspheres; however, the yield of the polymer was also low.

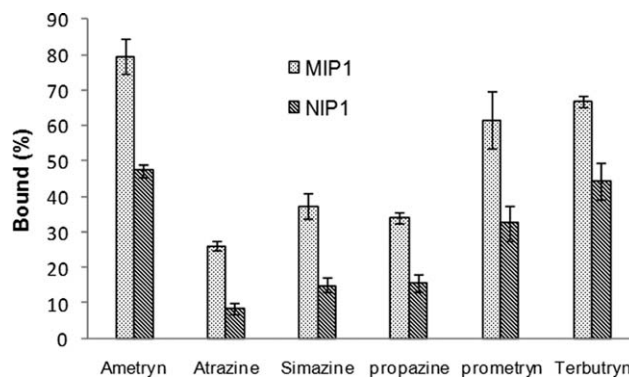
For the poly(4VPy-*co*-DVB) system, only neat acetonitrile was used as the reaction solvent. The polymer particles obtained in this solvent were uniform and had very narrow size distribution [Fig. 3(e,f)].

### Molecular binding characteristics of ametryn-imprinted polymers

The molecular imprinting effect was studied by comparing the uptake of ametryn by the imprinted polymers in comparison with the uptake of ametryn by the corresponding nonimprinted polymers. When acetonitrile was used as the binding solvent, the best molecular imprinting effect was observed for MIP1 (Figs. 4 and 5). Although MIP2 and MIP3 could bind 25–35% of ametryn, there was no obvious difference between the imprinted and the non-imprinted polymers (Fig. 4). The poor molecular imprinting effect for MIP3 may be explained by that 4VPy can only



**Figure 5** Uptake of ametryn by different amount of MIP1 (○) and NIP1 (□). Solvent: acetonitrile.



**Figure 6** Uptake of triazine herbicides by MIP1 and NIP1. Solvent: acetonitrile. Polymer conc.:  $10 \text{ mg mL}^{-1}$ . Initial conc. of herbicides:  $10 \text{ } \mu\text{g mL}^{-1}$ .

form weak interaction with ametryn through single hydrogen bond, which is different from MAA that is able to interact with ametryn through bidentate hydrogen bonds (Fig. 2). For MIP2, the apparently low cross-linking density may cause a high flexibility of polymer network and therefore poorly defined binding sites, which can explain the poor molecular imprinting effect.

### Selectivity of MIP1 for binding of triazine herbicides

The possibility of using ametryn-imprinted polymer to bind similar triazine herbicides was investigated for MIP1. In the equilibrium binding experiments, MIP1 and NIP1 ( $10 \text{ mg}$ ) were incubated with  $10 \text{ } \mu\text{g mL}^{-1}$  of six different triazine herbicides (ametryn, atrazine, simazine, prometryn, propazine, and terbutryn) in acetonitrile. The uptake of the different herbicides by the two polymers was measured by HPLC analysis. As shown in Figure 6, higher binding was observed for triazine herbicides containing the methylthio-group (ametryn, prometryn, and terbutryn) than that containing the chloro-group (atrazine, simazine, and propazine). These results suggest that the molecularly imprinted cavities match more closely the methylthio-moiety of the ametryn analogs than the smaller chloro group. In general, the imprinted polymer MIP1 displayed obvious specific binding for triazine herbicides that are structurally related to the ametryn template.

## CONCLUSIONS

In this work we have studied preparation of ametryn-imprinted polymer microspheres using three different functional monomers under precipitation polymerization condition. Uniform polymer beads were obtained using the three functional monomers in combination with DVB cross-linker under optimized solvent conditions. For the first time,

melamide was used as functional monomer to provide tridentate hydrogen bond interaction with the molecular template. The imprinted poly(melamide-co-DVB) displayed effective binding for ametryne, but the effect of imprinting was marginal. The MIP beads prepared using MAA as functional monomer showed the best specific binding for not only ametryne but also its structural analogs in the group of triazine herbicides tested. The optimal molecular imprinting effect achieved using MAA functional monomer can be explained by the hypothetical template-functional monomer complex. Considering their group selectivity for triazine herbicides and their very uniform physical morphology, the ametryne-imprinted poly(MAA-co-DVB) beads may be used as SPE adsorbents for routine herbicide analysis.

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