

Precipitation Polymerization of Molecularly Imprinted Polymers for Recognition of Melamine Molecule

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ABSTRACT: Molecular imprinted polymer microspheres (MIPs) were prepared by precipitation polymerization using melamine as template molecule, methacrylic acid (MAA) as functional monomer, trimethylol-propane trimethacrylate (TRIM) as crosslinking agent, acetonitrile as solvent and dispersion medium. Release of the template was performed by continuous extraction with methanol containing 10% acetic acid. The microspheres were observed by scanning electron microscopy (SEM). The perfect microspheres were produced when the addition of crosslinker was 7.48 mmol. The binding capacity of MIPs was examined, $Q_{\max} = 68.36 \mu\text{mol g}^{-1}$, and the dissociation constant

at binding site of MIPs, $K_d = 0.761$, was estimated. Compared with the performance of conventional imprinted polymer, the imprinted microspheres showed high selectivity in special binding to template molecule. The imprinted microspheres could be used as the stationary phase in HPLC or SPE for selective extraction of melamine in daily products. © 2011 Wiley Periodicals, Inc. *J Appl Polym Sci* 123: 962–967, 2012

Key words: molecular imprinted polymers; melamine; precipitation polymerization; molecular recognition; hydrogen-bonded complex

INTRODUCTION

Molecularly imprinted polymers (MIPs), three-dimensional network polymers, are prepared in the presence of template molecule. After the template molecules are removed, leaving cavities have selectively rebind template molecule on size, shape, and functionality.^{1–3} There is an increasing interest in the analytical of MIPs, which exhibit molecular recognition from amino acids, glucose, protein, to chiral analyze.^{4–8} Various MIPs have been applied in chemical sensors, chromatographic separation, SPE, and catalysis.^{2,9–11}

Now, there are many different developing methods widely used in preparing MIPs.^{12–16} The most used method is bulk polymerization, during which the template, monomer, crosslinker, and initiator are added into a glass tube and begin to polymerize initiated by thermal or photo (UV) radiation.^{17–19} The products need to be crushed, grinded, and then the particles with certain size are obtained. The cavities of the MIPs may be destroyed during the procedures of crush and grind; the irregular particles generally give less binding recognition in adsorption procedure. In the reported methods of

synthesizing melamine molecular imprinted polymers,^{20–22} precipitation polymerization are still rather rare now.²³ Compared with bulk polymerization, the precipitation polymerization, an especial method, is often used for preparing MIPs with uniform shape and low dispense phase distribution.^{23,24} The number of the nuclei, produced in the early stage of the polymerization, is found to be constant during the remainder of the polymerization. The nuclei grow to be monodisperse polymer particles without aggregation by capturing the other oligomers and monomers.²⁵ The particles obtained are monodispersed and can be directly used as the stationary phase in HPLC and SPE. The advantages of MIPs, such as stability at extremes of pH and temperature, ease of preparation, low cost, and reusability, have led to the development of various MIP applications in chromatographic separation and SPE. Melamine, tripolycyanamide, is an industrial chemical in the production of melamine resins, which are used in laminates, glues, adhesives, and plastics. He et al.⁴ have researched solid-phase extraction of melamine from aqueous samples using water-compatible molecularly imprinted polymers; Yu et al.²¹ have studied highly selective molecular recognition and high throughput detection of melamine based on molecularly imprinted sol gel film; Yang et al.¹⁷ have investigated molecularly imprinted polymer as SPE sorbent for selective extraction of melamine in dairy products. In this work, the MIPs with

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melamine as the template were synthesized by the precipitation polymerization. The morphology and the particle size were characterized by SEM, and the pore size was measured by nitrogen gas adsorption measurements. Moreover, the selectivity of MIPs was evaluated by the equilibrium rebinding experiment in solvent, and the MIPs showed a better function of separation and enrichment compared with existing melamine MIPs of bulk polymerization.¹⁷ To our knowledge, melamine MIPs can be used as selective SPE sorbents for the determination of melamine in dairy products.

EXPERIMENTAL

Materials

Methacrylic acid (MAA) was purchased from Tianjin Fuchen Chemical Reagent Plant (Tianjin), China and distilled to remove the polymerization inhibitor before use. Trimethylol-propane trimethacrylate (TRIM) was purchased from Tianjin Chemical Reagent Research Institute (Tianjin, China) and extracted three times with a mixture of aqueous NaOH (0.1M, 75 mL) and saturated NaCl (25 mL) and then one time with saturated NaCl solution (25 mL) to remove inhibitors. The washed TRIM was dried over magnesium sulfate and stored in a fridge. Azobis(isobutyronitrile) (AIBN) was the product of Beijing Chemical Works (Beijing), China and was recrystallized prior to use. Acetonitrile and methanol were obtained from Tianjin Tiantai finechemical (Tianjin, China) system.

Preparation and characterizations of the MIPs microspheres

Synthesis of the melamine imprinted microspheres was carried out with 0.4 mmol melamine, 4.9 mmol MAA, 2.5 mmol AIBN, and different volume TRIM in 40mL acetonitrile in a 100 mL three-necked flask. The solution was treated with ultra-sound for 15 min and then was purged with nitrogen flow for 10 min. Polymerization was carried out in a water bath at 60°C for 24 h. The obtained microspheres were collected by centrifuge. The product was extracted with ethanol containing 10% acetic acid using a Soxhlet extractor for 48 h. The microspheres were finally rinsed with acetone and then dried in an oven 45°C for 24 h before use. As a contrast, the nonimprinted microspheres were prepared and treated in exactly the same way except that the template was absent in the polymerization procedure.

The feature of MIPs were characterized by scanning electron microscopy (SEM) (JEOL JSM 6700F) and the specific surface area of the particles was determined by nitrogen gas adsorption measure-

ments using BET (Micromeritics Tristar-3000). Evaluation of the binding ability 5 mg of the microspheric polymer was put into a 10 mL calorimetric tube. Then 5 mL of melamine acetonitrile solution with known concentration was mixed with the polymer. The mixture was slightly shaken on a horizontal shaker for 24 h at room temperature. After centrifugation and careful separation of the polymer microspheres, the solution was filtered through a membrane filter. The concentration of the melamine after adsorption by the polymer was determined by measuring the solution absorbance at 238 nm. The average binding percentage was calculated with nine repeated binding processes. UV spectra were taken on a Puxi UV-1810s type absorption spectrometer.

RESULTS AND DISCUSSION

The template molecule, melamine, is a triazines nitrogen-containing heterocyclic compound containing amide group. The chemical name is 1,3,5-Triazine-2,4,6-triamine. The melamine has three $-\text{NH}_2$ groups which easily form noncovalent with function molecule. Obviously, the binding site is located at the point of $-\text{NH}_2$ group on the melamine with carboxyl of MAA through hydrogen bond. In theory,²⁶ template molecule (A) and functional molecules (B) form a complex that can be shown as follows:



where K is binding constant, $n = 1, 2, 3, \dots$. The concentration of functional monomer (b_0) is much larger than that of template molecule (a_0), so the concentration of complex (c) can be expressed as:

$$c = \frac{a_0 b_0 k}{1 + b_0^n k} \quad (2)$$

At a particular absorption wavelength, the absorbance of the three substances in the solution is A .

$$A = (a_0 - c)\xi_A l + c\xi_C l + (b_0 - c)\xi_B l \quad (3)$$

Where consider ξ_A and ξ_C are molar extinction coefficient of the template A and complexes C , respectively.

$$\Delta A = A - a_0 \xi_A l - b_0 \xi_B l = c \Delta \xi l \quad (4)$$

$$\Delta \xi = \xi_C - \xi_A - n \xi_B \quad (5)$$

$$\frac{\Delta A}{b_0^n} = -k \Delta A + k \Delta \xi a_0 l \quad (6)$$

Mapping of ΔA to $\Delta A/b^n$, the value n could be derived, so that the role model of the template molecule and functional monomer in acetonitrile was

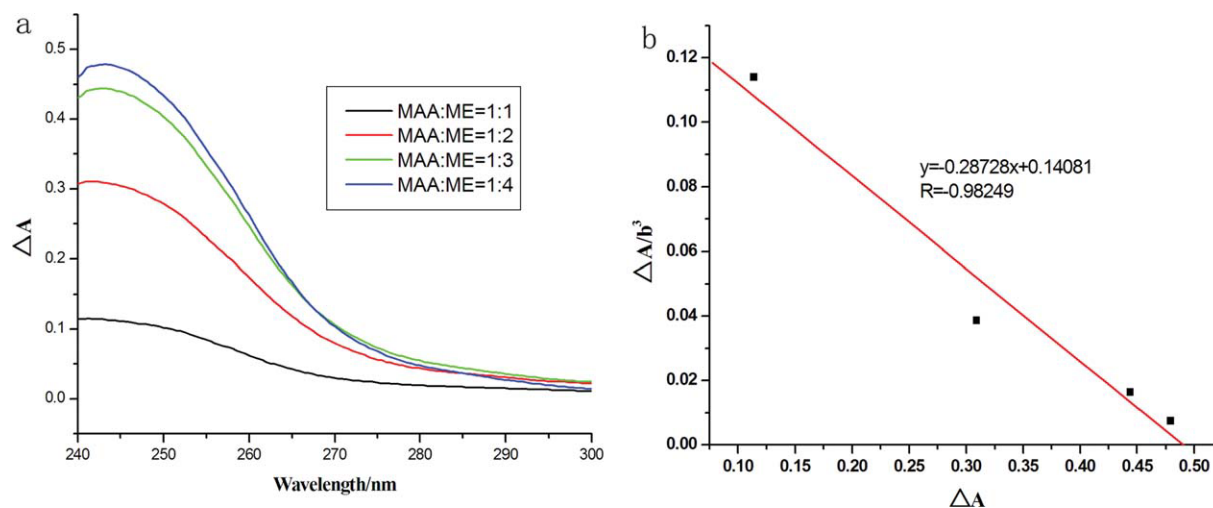


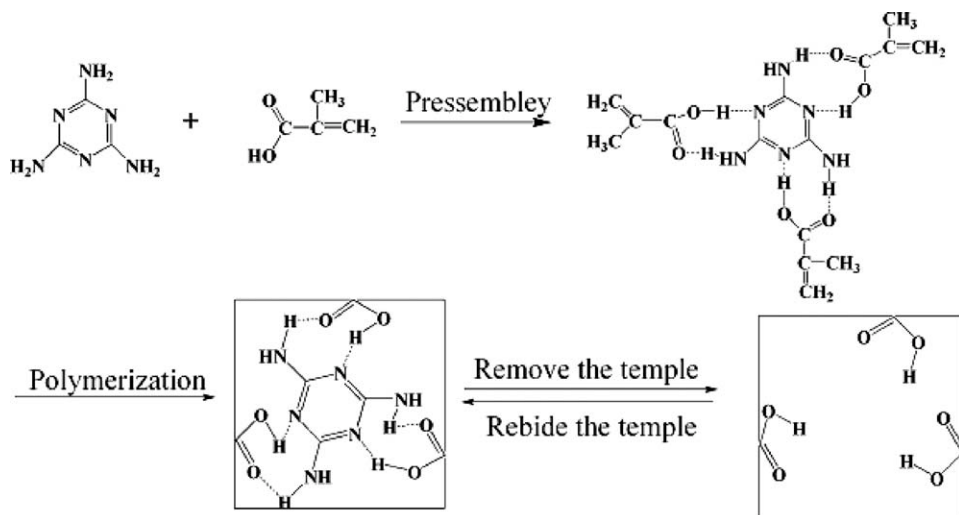
Figure 1 a: Adsorption spectra of melamine in the presence of MAA; b: Relationship between $\Delta A/b^3$ and ΔA at 242 nm $b = 1,2,3,4$. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

launched, which revealed the mechanism of molecular imprinting. Figure 1 showed that the interaction ratio between MAA and Melamine is 3 : 1, that is, one melamine molecule and three MAA molecules will interact to form one complex. The complex of functional monomers in the vicinity of the melamine molecule is considerably stable and followed by copolymerization with crosslinking monomers to form a polymer network. To remove the melamine molecule from MIPs after crosslinking, it is necessary to break the hydrogen bond between melamine molecule and carboxyl group of the polymer. The general mechanism for the formation of specific binding sites in noncovalently formed MIPs is outlined in Scheme 1.

The MIPs applied in the determination of melamine were prepared using melamine as template molecule. With acetonitrile as solvent and dispersion

medium, melamine as template molecule, MAA as functional monomer, and TRIM as crosslinking agent, molecularly imprinted microspheres were synthesized at certain experimental conditions. The effect of addition of crosslinker on shape and size of polymer are summarized in Table I.

From Table I, it can be seen that with different addition of crosslinker in polymerization, polymers can have various physical configurations. With increased addition of crosslinker, the shape of polymer particles became regular spheres. In Figure 2, both imprinted microspheres (MIPsB) and nonimprinted amorphous particles (NMIPsA) were polymerized with the same TRIM concentration, except different melamine addition. In the absence of template molecule, the nonimprinted polymers were conglobated together, it was because that the polarity of acetonitrile is strong, the hydrophobic repulsion



Scheme 1 Outline of the molecular imprinting process for melamine.

TABLE I
Polymer Configuration and Size Under Different
Polymerization Conditions

| Entry | Melamine (mmol) | TRIM (mmol L ⁻¹) | Configuration | Size (nm) |
|--------|--------------------|---------------------------------|---------------|-----------|
| NMIPsA | 0 | 5.61 | Monolith | |
| MIPsB | 0.4 | 5.61 | Microsphere | 400–900 |
| MIPsC | 0.4 | 7.48 | Microsphere | 600–700 |
| MIPsD | 0.4 | 9.36 | Microsphere | 400–1000 |

between function molecule MAA and solvent would become strong, which easily resulted in the formation of particle aggregations,²⁷ and this result is similar with the report.⁷ The perfect microspheres were produced when the addition of crosslinker is 7.48 mmol. The concentration of the crosslinker affects the morphology and size of MIPs, even if the concentrations of template and monomer didn't change. Copolymerization carries out at low concentrations of the whole bag of monomer, and microspheres are formed in a large number of media. In the case of higher concentration of TRIM in the polymerization process, acetonitrile dispersed into the polymer and the organic

phase reduced relatively. The microspheres generated with uneven size and rough surface.⁷

The data of surface area, pore size, and volume measured were listed in Table II. Table II indicated that MIPsD possessed the largest surface area due to higher addition of crosslinker (TRIM), while NMIPsA possessed the smallest surface area. Specific surface area, pore size, and volume of the MIPs were higher than those of NMIPs, which resulted in the higher rebinding capacity of the MIPs than that of NMIPs. When the polymerization occurred and solid polymer appeared, the solvent, acetonitrile as porogen, was absorbed. When the solvent volatilized, interconnect pores were formed inside of the polymers. And when the template molecule was removed, more special adsorption cavities were leaved, which contained precise arrangement of functional groups that provided by the functional monomer, matched with functional groups of template molecule. As shown in the Table II, the pore size was increased with the increasing crosslinker. When the concentration of crosslinker was 9.36 mmol, larger size pores were formed, and the highly rigid structure had an effect on the pores size. It

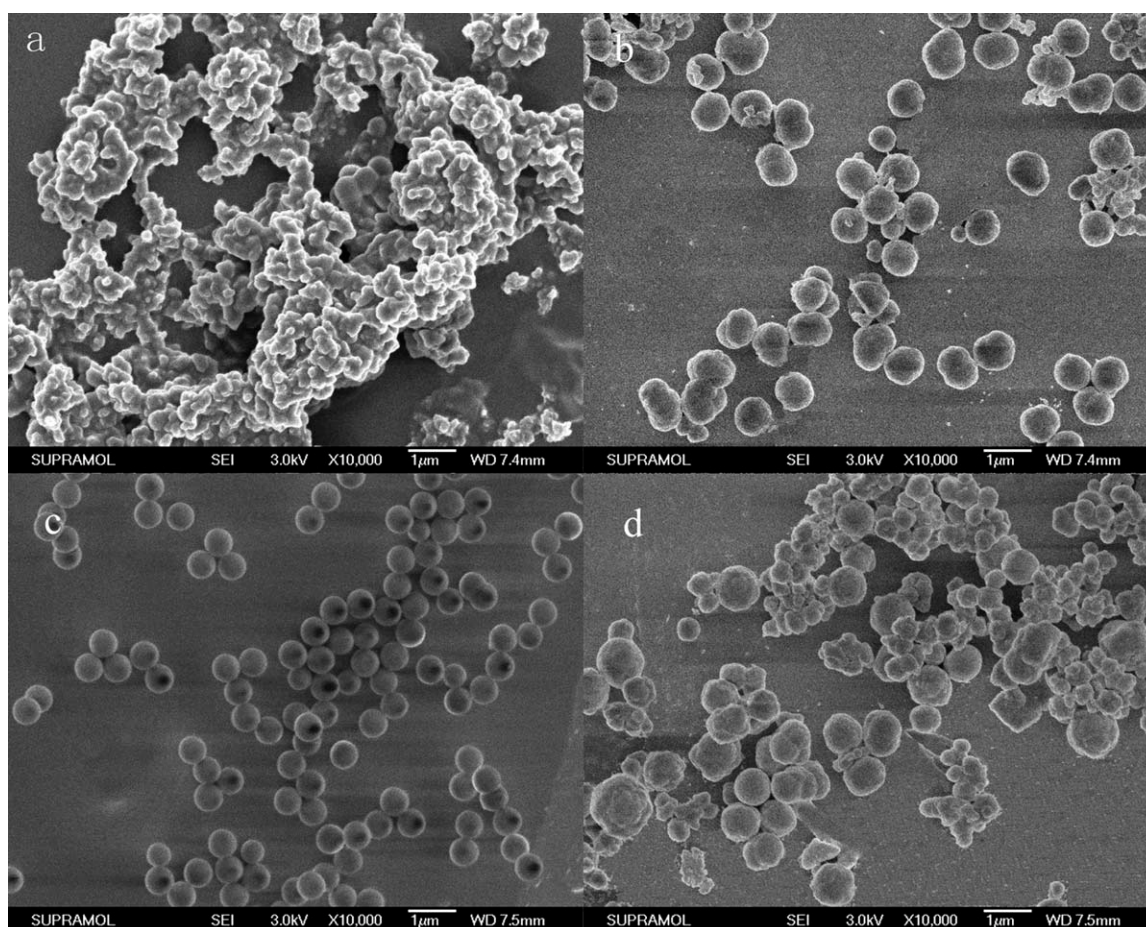


Figure 2 SEM of effect of crosslinker concentration on the configuration of the polymer a-NMIPsA; b-MIPsB; c-MIPsC; d-MIPsD.

TABLE II
Results of BET Characterization

| Polymer | Specific surface area (m ² g ⁻¹) | Pore volume (cm ³ g ⁻¹) | Pore diameter (nm) |
|---------|---|--|--------------------|
| NMIPsA | 20.4103 | 0.051957 | 10.1825 |
| MIPsB | 25.3825 | 0.086754 | 12.8392 |
| MIPsC | 36.9562 | 0.32716 | 24.3296 |
| MIPsD | 57.7831 | 0.534674 | 37.0125 |

could be interpreted as follows, the more rigid the polymer structure and the trifunctional structure of TRIM, the easier it is to keep the desired three dimensional structure and the affinity sites with desired three dimensional structures.²⁷

Macromolecular contributions to molecular recognition in MIPs include incorporation of the carboxyl group in the crosslinker backbone which not only reduces the conformational flexibility of the binding site, but also reduces the entropic effect associated with binding interactions.¹² Binding performance of molecularly imprinted polymers for melamine was studied by using a static adsorption method. Adsorption capacities of MIPs for melamine were evaluated by static equilibrium adsorption quantity Q which was calculated according to the following equation:

$$Q = \frac{(C_0 - C) \times V}{m}$$

Where Q is equilibrium adsorption quantity of MIPs ($\mu\text{mol g}^{-1}$), C_0 is the initial melamine concentration ($\mu\text{mol L}^{-1}$); C the final melamine concentration ($\mu\text{mol L}^{-1}$); V the volume of test solution (mL); the mass of dried polymer (mg). Melamine concentration was

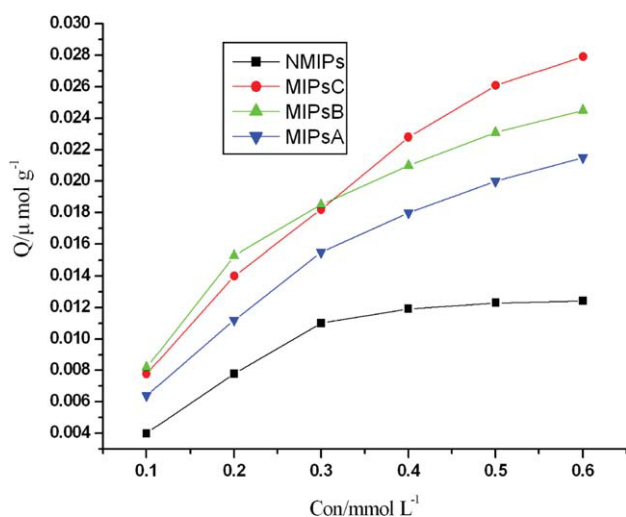


Figure 3 The binding isotherm of MIPs for melamine MIPs and NMIPs at 25°C. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

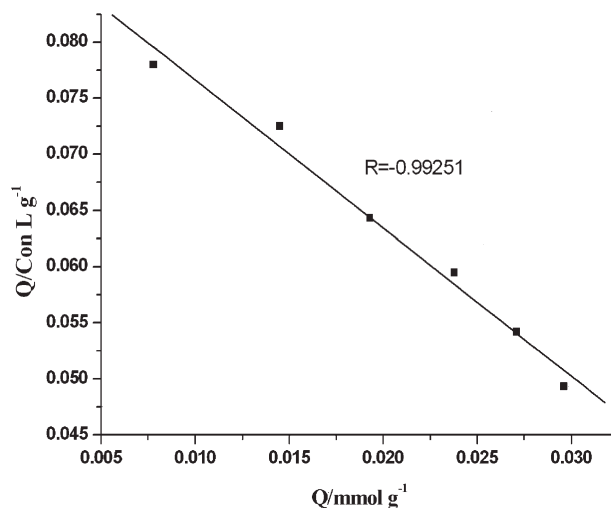


Figure 4 Scatchard plot analysis of the binding of melamine onto the MIPs.

calculated by measuring melamine absorbance in the supernatant with UV detection. The dissociation constants of the polymers were calculated by Scatchard equation:²⁸

$$\frac{Q}{C} = \frac{Q_{\max} - Q}{k_d}$$

Figure 3 showed that the change of Q values of MIPs and NMIPs tended to flat at with a rise in the concentration of melamine. However, the disparity in Q values of them became larger with the increasing concentration of melamine. This indicated that the cavities structure of them had obvious difference and suggested that melamine binds to the MIPs more possibly due to the specific binding to a limited number of binding sites than due to the nonspecific adsorption to the NMIPs network.²³ When the concentration of crosslinker was 9.36 mmol, larger size pores were formed and perfect adsorption line was given. When the proportion of MAA and TRIM was lower, the functional groups were so close with each other that they could not work independently. As could be seen from Figure 4, the Scatchard plot for MIPs was an excellent linear curve. The linear regression equation for this curve was $Q/C_{\text{on}} = 0.08983 - 1.31397Q$. The K_d and Q_{\max} were calculated to be 0.761 and 68.36 $\mu\text{mol g}^{-1}$ of dry polymer, respectively. Compared with the performance of conventional imprinted polymer, the MIPs showed a better function of separation and enrichment.¹⁷

CONCLUSIONS

The MIPs particles for melamine were prepared by precipitation polymerization and the MIPs showed specific affinity toward melamine in the binding

recognition process. The results showed that the interaction ratio between MAA and Melamine was 3 : 1. The BET results indicated that formation more porogen of the particles with higher addition of crosslinker. The result showed that the disassociation constant at binding site, K_d , equal to 0.761.

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