

Synthesis of Glutathione Imprinted Polymer Particles *via* Controlled Living Radical Precipitation Polymerization^{*}

Ren-yuan Song^{**}, Xiao-ling Hu, Ping Guan, Ji Li, Li-wei Qian and Qiao-li Wang
School of Natural and Applied Science, Northwestern Polytechnical University, Xi'an 710072, China

Abstract A general protocol was described for fabricating uniform molecularly imprinted polymer (MIP) particles *via* controlled living radical precipitation polymerization at ambient temperature. By adopting glutathione as model template, benzyl dithiocarbamate as iniferter agent, 4-vinylpyridine as monomer, and ethylene glycol dimethacrylate as cross-linker, it is demonstrated that the polymerization parameters including the iniferter concentration, monomer loading and molar ratio of cross-linker to functional monomer have profound effect on the final particle size and recognition property of the MIP particles. The batch static binding experiments were carried out to estimate the adsorption kinetics, adsorption isotherms and selective recognition of the MIP particles. The adsorption behavior followed the pseudo-second order kinetic model, revealing that the process was chemically carried out. Two adsorption isotherm models were applied to analyze equilibrium data, obtaining the best description by Langmuir isotherm model. In addition, the MIP particles also could selectively recognize glutathione over similar analogs, indicating the possibility for the separation and enrichment of the template from complicated matrices.

Keywords: Molecularly imprinted polymer; Controlled living radical polymerization; Particle size; Recognition; Glutathione.

INTRODUCTION

Glutathione (GSH, 5-L-glutamyl-L-cysteinylglycine) is a tripeptide with three amino acid residues^[1–3]. Several methods, including spectrofluorometry^[4], capillary electrophoresis^[5] and chromatography^[1], have been presented to analyze GSH in biological matrices. Although such methods offer more sensitive approaches to GSH analysis, relatively expensive instruments, which are not yet available in all laboratories, are required. In addition, most of the reported determination methods have difficulty in direct separation and enrichment of GSH from complex matrices through sample pretreatment. Generally, GSH is present in complex samples at low concentrations, thus, developing a simple and novel determination method appropriate for GSH is necessary.

Molecular imprinting is a generic approach to the synthesis of three-dimensional cross-linked polymeric materials with specific binding sites for a target molecule^[6–7]. These binding sites are tailor made *in situ* by the copolymerization of functional monomer, cross-linker in the presence of a target molecule and suitable porogenic solvent. When the target molecule is removed, a highly specific imprinting cavity is formed within the rigid network polymer matrix, which has high binding affinity towards the target molecule^[8]. Recently, molecularly imprinted polymers (MIPs) particles have been used for biologically interesting targets including peptides and proteins. These particles have outstanding molecular recognition properties and high stability that enable applications in biology and separation of targets from samples^[9].

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^{**} Corresponding author: Ren-yuan Song (宋任远), E-mail: songrenyuan0726@163.com

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The methods commonly used to prepare MIP particles mainly including emulsion polymerization^[10], suspension polymerization^[11], dispersion polymerization^[12], precipitation polymerization^[13] and so on. Among the different approaches currently used to prepare MIP particles, precipitation polymerization has unique advantages such as mild conditions, simple process and general applicability in various molecular-imprinting systems^[14], especially in peptide-imprinting systems^[15]. However, conventional precipitation polymerization cannot accurately control polymer architecture and morphology owing to the use of traditional free-radical polymerization (TFRP)^[13–15, 7].

Currently, MIP particles prepared by TFRP are difficult to be controlled owing to side reactions with impurities and chain transfer reactions, which lead to the formation of polymer networks with heterogeneous structures^[16]. The presence of heterogeneity within the network structures of MIPs can significantly affect the binding sites created inside them, which may be related to the intrinsic drawback of MIPs such as broad binding site heterogeneity, relatively low binding affinity and low selectivity^[17]. As a result, the synthesis of MIP particles by TFRP is greatly limited. Thus, the propagation and termination steps of the reaction should be controlled. The introduction of controlled living radical polymerization (CRP) into precipitation polymerization systems can considerably improve the homogeneous network structures of MIP particles and elucidate their structure-property relationship.

CRP is a very effective tool for controlling cross-linked polymer structures. Thus far, many different polymer networks with homogeneous structures have been prepared by CRP. Iniferter is an ideal candidate for CRP because of its versatility and simplicity. Iniferter, an initiator-chain transfer molecule, was first discovered by Otzu *et al.*^[18] in 1982. Since then, iniferter has been used to produce linear polymer chains with low polydispersities, specific block copolymers, graft polymers and cross-linked polymer systems on surfaces^[19–24]. To date, several successful applications in the molecular imprinting field have been reported. For example, Su and coworkers^[25] have prepared sulfamethazine-imprinted core-shell microspheres by surface-initiated iniferter controlled living radical polymerization (ICRP) on silica microspheres. These resultant core-shell MIPs showed high affinity toward the template. Furthermore, the target molecule was detected from complicated samples. The same method was used by Barahona and coworkers^[26]. They immobilized iniferter agent on silica particles and synthesized thiabendazole-imprinted particles by surface ICRP. The formation of MIP particles with narrow particle size distribution and core-shell morphology can be attributed to the intrinsic characteristics of ICRP. Very recently, Zhang and coworkers^[27] reported that ICRP combined with precipitation polymerization can improve the binding and structural characteristics of MIP particles. Nevertheless, these studies focused on the formation of MIPs on surfaces *via* using ICRP. By contrast, few studies have been conducted on the effects of preparation conditions on the particle size distribution, network structures and morphology of the MIPs. And these technology parameters have great influence on the recognition properties of the MIP particles.

In this present study, we started to investigate the control of morphologies and recognition properties of MIP particles that can be synthesized by iniferter controlled living radical precipitation polymerization (ICRPP) technique at ambient temperature. During the polymerization, the polymerization parameters including the iniferter concentration, monomer loading and molar ratio of cross-linker to functional monomer used not only affected the final particle size but also the specific molecular recognition of the MIP particles. The particle size and morphology of MIP particles were characterized using scanning electron microscopy (SEM). The recognition properties of MIP particles were analyzed with a batch static adsorption experiments. In addition, under the optimal conditions, the adsorption properties of the resulting MIP particles including kinetics, isotherms and selectivity have been discussed in detail.

EXPERIMENTAL

Materials

GSH, oxidized GSH (GSSG), and glycyl-glycine (Gly-Gly) were purchased from Shanghai Jingchun Ke Fine Chemical Corporation. 4-Vinylpyridine (4-VP) and ethylene glycol dimethacrylate (EGDMA) were purchased

from Alfa. They were distilled under a vacuum to remove the inhibitor. Tetramethylammonium hydroxide, phenylisothiocyanate (PITC), high-performance liquid chromatography (HPLC)-grade acetonitrile and triethylamine were provided by Sinopharm Chemical Reagent Co., Ltd. All chemicals were of analytical grade and used without further purification. Benzyl dithiocarbamate (BDC) was prepared following a literature procedure^[28].

Synthesis of Glutathione Imprinted Polymer Particles

Glutathione imprinted polymer particles were synthesized according to Zhang *et al.*^[27]. In a typical synthesis, a mixture of H₂O-MeCN (20 mL; 1/9, *V/V*) was introduced into the reactor as the reaction medium. Then, GSH (51.2 mg, 0.167 mmol), 4-VP (0.11 mL, 1 mmol), EGDMA (0.75 mL, 4 mmol), and BDC (35.85 mg, 0.15 mmol) were sequentially added to a four-neck round-bottom quartz flask (100 mL). After sealing, mixing and sparging the mixture with nitrogen for 30 min, polymerization was initiated by ultraviolet radiation from a high-pressure mercury lamp (300 W, 365 nm) with stirring at ambient temperature for 12 h. After polymerization, the resulting polymer was extracted with ethanol containing 10% acetic acid using a Soxhlet apparatus for 48 h. Finally, the obtained polymer was washed with ethanol and water three times and then dried in a vacuum at 40 °C for 36 h.

Corresponding non-imprinted polymer (NIP) particles were prepared and purified under the same conditions but without template addition.

Characterizations

The chemical structures of the obtained polymer were identified by Fourier-transform infrared spectrometer (FTIR; Shimadzu, WQF-310) using the KBr compressed pellet method. The particle size and size distribution of the MIPs were determined with SEM (FEI, Quanta 600FEG). All of the SEM size data reflect the averages of about 100 particles each, which are calculated by the following formula (1–3):

$$U = D_w / D_n \quad (1)$$

$$D_n = \sum_{i=1}^k n_i D_i / \sum_{i=1}^k n_i \quad (2)$$

$$D_w = \sum_{i=1}^k n_i D_i^4 / \sum_{i=1}^k n_i D_i^3 \quad (3)$$

where U is the polydispersity index, D_n is the number-average diameter, D_w is the weight-average diameter, k is the total number of the measured particles, n_i is the particles number of the determined microspheres, and D_i is the particle diameters of the determined microspheres; the values of D_i and n_i were determined by the Nano Measurer 1.2.5 software.

Static Adsorption Experiments

The static adsorption experiments were carried out to estimate the adsorption kinetics and adsorption isotherms at 25 °C. In a typical procedure, 10 mg of MIPs or NIPs was incubated with 10 mL of 0.5 mg·mL⁻¹ GSH solution (pH 7.0) for different times. After filtration through acetate membrane (0.45 μm pore size), the residual concentrations of GSH were determined by HPLC. And the adsorption capacity Q_t (mg·g⁻¹) is calculated according to the following formula (4):

$$Q_t = \frac{(c_0 - c_t)V}{m} \quad (4)$$

where c_0 (mg·mL⁻¹) and c_t (mg·mL⁻¹) represent the initial and residual concentrations, respectively. V (mL) is the volume of adsorption solution, and m (g) is the weight of the adsorbent.

Equilibrium adsorption experiments were carried out by incubating MIPs or NIPs with different concentrations of GSH solution for 400 min. The equilibrium adsorption capacity Q_e (mg·g⁻¹) is calculated according to the following equation (5):

$$Q_e = \frac{(c_0 - c_e)V}{m} \quad (5)$$

Selectivity Experiments

GSSG and Gly-Gly (Fig. 1) were selected as interferents because of their similar structure with GSH to evaluate the binding selectivity of MIPs towards GSH. In a typical procedure, a mixed solution of GSH/GSSG/Gly-Gly was prepared and the concentration of the three substances in the mixed solution were all kept as $0.5 \text{ mg} \cdot \text{mL}^{-1}$. 50 mg of MIPs or NIPs was incubated with 10 mL of the above mixed solution at 25°C for 400 min. After reaching adsorption equilibrium, the concentrations of GSH, GSSG and Gly-Gly in the supernatant were determined by HPLC. The distribution and selectivity coefficients of GSSG and Gly-Gly with respect to GSH can be obtained from the static adsorption experiments data according to following parameters:

distribution coefficient:

$$K_d = \frac{Q_e}{c_e} \quad (6)$$

selectivity coefficient:

$$k = \frac{K_{d(\text{template})}}{K_{d(B)}} \quad (7)$$

relative selectivity coefficient:

$$k' = \frac{k_{\text{MIPs}}}{k_{\text{NIPs}}} \quad (8)$$

where Q_e and c_e are the equilibrium adsorption capacity ($\text{mg} \cdot \text{g}^{-1}$) and equilibrium concentration ($\text{mg} \cdot \text{mL}^{-1}$), respectively; $K_{d(\text{template})}$ and $K_{d(B)}$ are the distribution coefficient of GSH and its similar structural compounds (GSSG and Gly-Gly), respectively; k_{MIPs} and k_{NIPs} are the separation factors of the MIPs and NIPs, respectively.

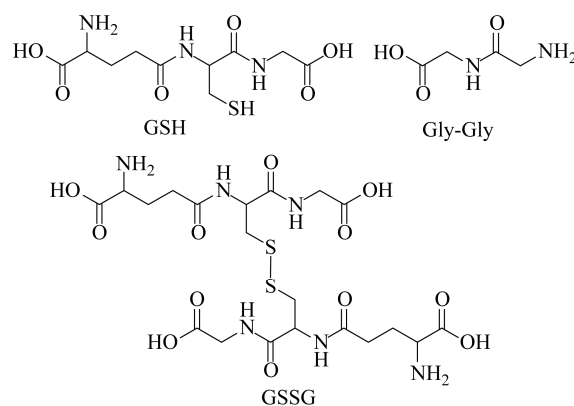


Fig. 1 Chemical structures of GSH and its analogs

RESULTS AND DISCUSSION

The general scheme for the synthesis of MIP particles is illustrated in Fig. 2. As we known, template molecule (GSH) has both amino and carboxylic acid groups in its structure, and it is an acid template molecule ($\text{PI} = 5.93$). In general, for an acid template molecule, we select alkaline molecule monomer as functional monomers, such as vinylpyridine and vinylimidazole. Dinçer and coworkers^[29] have successfully prepared glutathione-imprinted polymers using alkaline 1-vinylimidazole molecule as functional monomer in the imprinting process. In this study, we choose 4-VP as the functional monomer which has two merits: first, the strong interactions are formed

between the acid template molecule and alkaline functional monomer; secondly, 4-VP is only proton acceptor instead of electron donor, thus, it can be effectively reduced the number of non-specific binding sites in the polymerization process. In addition, BDC was used as iniferter agent in an attempt to improve recognition properties of MIP networks in the precipitation polymerization systems. Hence, considering iniferter characteristics, the obtained MIPs can be expected to have uniform particle size distribution and high specific recognition performance when prepared by ICRPP. A model non-covalent molecular imprinting system was selected to show proof-of-principle. This system utilized GSH, 4-VP, EGDMA and an acetonitrile-water mixture as the template, functional monomer, cross-linker and porogenic solvent, respectively.

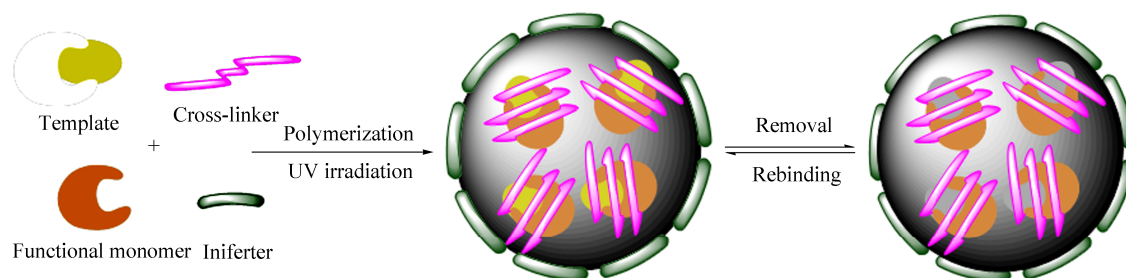


Fig. 2 General scheme for the preparation of MIP particles and their use for molecular recognition (Template molecules induce the formation of binding sites during the ICRPP.)

Control over the size uniformity of the MIPs particles is highly important for their potential applications and is the major area of research interest. Thus, the effects of various polymerization parameters on the morphologies and recognition properties of the obtained polymer particles prepared by ICRPP were studied. These properties were iniferter concentration, monomer loading and molar ratio of EGDMA to 4-VP. The morphologies of the obtained MIP particles were analyzed by SEM. The binding capacities of the obtained MIPs or NIPs particles were determined by equilibrium binding experiments. SEM images showing the morphologies of the obtained MIP particles (samples M1 to M7 as defined in Table 1) are displayed in Fig. 3. Measurement data are listed in Table 1.

Table 1. Synthesis and characterization data for MIPs particles prepared by ICRPP of 4-VP, BDC and EGDMA under different reaction conditions

Entry	Iniferter (wt%)	4-VP+EGDMA (vol%)	EGDMA/4-VP (molar ratio)	D_n (μm)	D_w (μm)	U	Q_{MIPs}^a ($\text{mg}\cdot\text{g}^{-1}$)	Q_{NIPs}^a ($\text{mg}\cdot\text{g}^{-1}$)	α^b
M1	1.5	5 %	4:1	0.76	0.90	1.184	10.41	4.87	2.13
M2	3	5 %	4:1	0.83	0.89	1.080	12.70	5.16	2.46
M3	4.5	5 %	4:1	0.87	0.95	1.096	13.54	5.83	2.32
M4	1.5	3 %	2:1	0.79	0.93	1.181	8.46	4.35	1.94
M5	1.5	5 %	2:1	1.28	1.45	1.130	9.08	4.48	2.02
M6	1.5	10 %	2:1	1.49	1.95	1.312	11.85	6.01	1.97
M7	1.5	5 %	8:1	0.83	0.89	1.082	6.15	3.94	1.56

^a Q_{MIPs} and Q_{NIPs} are the adsorption capacities of MIPs and NIPs, respectively.

^b Imprinting factor, $\alpha = Q_{\text{MIPs}}/Q_{\text{NIPs}}$.

Effect of Iniferter Concentration

An important standard for the selection of appropriate initiator is that it should be able to control the polymerization rate and size uniformity of the resulting MIPs. Thus, to investigate the effect of iniferter concentration on the morphology and recognition property of MIPs particles, a series of different BDC concentrations was used while fixing other polymerization conditions. The SEM images of the MIP particles with different BDC concentrations are shown in Fig. 3. The images demonstrated that the resulting MIPs had a spherical shape with a smooth surface. The size and size distribution of the MIP particles are shown in Table 1 (entries M1–M3) with different BDC levels. The size of the MIP particles increased from 0.76 μm (entry M1) to

0.87 μm (entry M3) with increased BDC concentration from 1.5 wt% to 4.5 wt%, similar to previously observed precipitation polymerization systems^[19]. This finding can be ascribed to the formation of a number of free radicals with increased BDC concentrations. Thus, increasing the concentration of growing oligomers resulted in fewer mature nuclei formed, which led to larger final particles^[30]. However, the size of distribution of the MIP particles did not change in one direction, and the narrowest distribution was obtained at a BDC concentration of 3 wt% with a size distribution index of 1.080 (entry M2). The other BDC concentrations with more or less BDC produced particles with broader distributions. This finding may be explained by the fact that the nucleation stage was prolonged in the ICRPP system because a high molecular weight was not instantaneously formed, resulting in broad particle size distributions^[31].

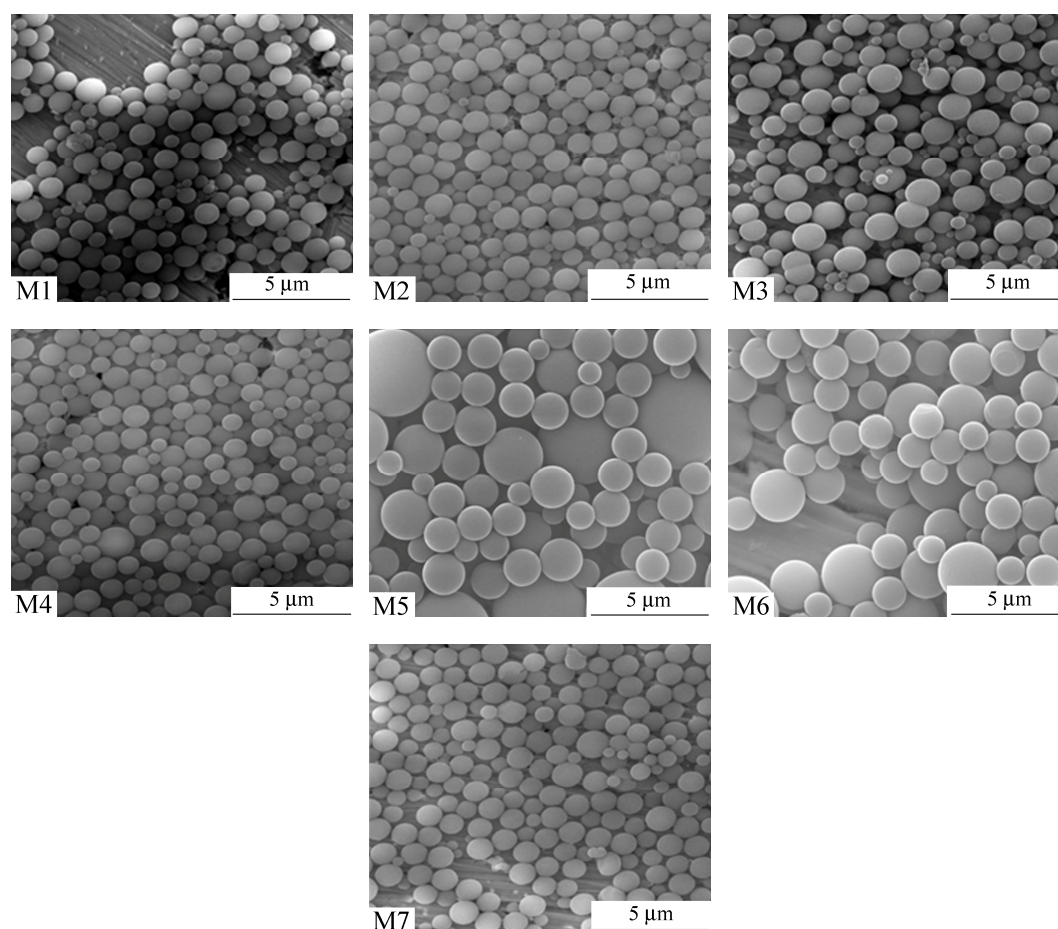


Fig. 3 SEM images of MIPs synthesized under different experiment conditions including iniferter concentration, monomer loading, and molar ratio of EGDMA to 4-VP (Samples M1–M7 correspond to entries M1–M7 in Table 1, respectively.)

With increased BDC concentration, the imprinting factor did not change in one direction, the M2 showed the highest imprinted factor (2.46) when the BDC concentration was 3 wt%. The variation in imprinted factors with altered BDC concentration can be explained as follows. The ICRPP technique involved the introduction of chain-transfer agents that combined with the polymer radical to undergo a reversible termination reaction, thereby forming a meta-stable dormant species. The result was extended propagation period of the polymerization reaction. This longer propagation allowed the polymer chains to grow uniformly and simultaneously, resulting in more monodispersed polymer chains and more homogenous polymer networks^[32],

which benefits for formation of structural homogeneity of specific recognition binding sites in the polymerization process. In addition, the M2 has high imprinting factor probably due to the narrower size distribution affected recognition property in the binding process. Hence, adjusting the BDC concentration increased the specific recognition property in the ICRPP system, thereby allowing the creation of MIPs particles with tailorable binding parameters. So the BDC concentration of 3 wt% was chosen as the optimized condition for GSH imprinting.

Effect of Monomer Loading

As shown in the SEM images in Fig. 3, the MIPs particles had spherical shapes and smooth surfaces with different monomer loadings. Furthermore, the size and size distribution of the MIP particles with different monomer loadings ranged from 3 vol% (entry M4) to 10 vol% (entry M6), are shown in Table 1. With increased monomer loading, the size of the MIP particles dramatically increased from 0.79 μm to 1.49 μm , whereas the size distribution index increased from 1.181 to 1.312. This phenomenon has been observed in traditional precipitation polymerization systems^[33, 34] and can be the result of the increase in the solubility of the growing polymer chain owing to the formation of template-polymer complexes. These complexes allow the template-polymer compound with relatively higher molecular weights to remain soluble in the reaction media, leading to decreased amount of particle nuclei, and thus, larger particle sizes^[7, 31]. In addition, polydispersed MIPs particles were obtained probably due to the higher reaction rate caused by the more active centers in the polymerization mixture with higher monomer loadings, which resulted in much larger size and wider size distribution.

In same conditions, with the increase of monomer loadings from 3 vol% to 10 vol%, the imprinting factor of the MIP particles increases firstly (entry M5) and then decreases (entry M6), so that gets an optimum (5 vol%, entry M5). This finding can be attributed to the higher growing polymer chain and phase separation rate that influenced the nucleation and growth process of the particles^[7]. In fact, the increase in specific recognition property of MIPs can be ascribed to the decrease in kinetic chain length caused by iniferter, which led to increased structural homogeneity of MIPs with more stability and integrity of more appropriately sized binding sites^[34]. However, the M5 has high imprinting factor (2.02) probably because the narrower size distribution affected recognition property in the binding process. Therefore, 5 vol% of monomer loading was adopted as the optimal concentration in the subsequent adsorption experiments.

Effect of Molar Ratio of EGDMA to 4-VP

Figure 3 shows the SEM images of the MIP particles with different ratios of EGDMA to 4-VP (entries M5, M1, and M7). The size and the size distribution of the MIP particles with different ratios of EGDMA to 4-VP ranging from 2:1 (entry M5) to 8:1 (entry M7) are listed in Table 1. The polydispersed MIP particles were obtained when the ratios of EGDMA to 4-VP ranged from 2:1 to 4:1, whereas the monodispersed MIP particles were formed at 8:1 EGDMA-to-4-VP ratio. This finding may be due to the fact that the lower ratio of EGDMA to 4-VP delayed nucleus formation and particle nucleation, resulting in particle sizes with a broader distribution. Moreover, the size of the MIP particles significantly decreased from 1.28 μm at 2:1 (entry M5) ratio of EGDMA to 4-VP to 0.76 μm at 4:1 (entry M1) ratio of EGDMA to 4-VP, similar to the results of Huang *et al.*^[31]. This finding may be explained by the formation of a larger number of nuclei with increased ratio of EGDMA to 4-VP at the initial stage, which decreased the particle size. Surprisingly, when the ratio of EGDMA to 4-VP was 8:1 (entry M7), the size of the MIP particles increased to 0.83 μm . The variation in size of the MIP particles can be attributed to the increase in solubility of the initially formed branched oligomers with increased ratio of EGDMA to 4-VP, which allowed the oligomers with relatively higher molecular weights to remain soluble in the reaction media. This phenomenon led to a decrease in the amount of particle nuclei, and thus, larger particle sizes^[35].

The imprinting factors of the MIP particles with different molar ratios of EGDMA/4-VP are illustrated in Table 1. With increasing ratio of EGDMA to 4-VP to 4:1 (entry M1), the MIP particles exhibited higher imprinting factor. With increased molar ratio of EGDMA to 4-VP to 8:1 (entry M7), the imprinting factor was drastically decreased. The variation in binding properties with different molar ratios of EGDMA to 4-VP can be explained as follows. In the imprinting process, the template binding properties strongly depended on the

network structure of imprinted polymer networks. Compared with M5, M1 had higher specific recognition property because of the high cross-linking degree that could have reduced the swelling and maintain the stability of recognition cavities^[36]. Nevertheless, the excessive high cross-linking degree (entry M7) often led to small pore sizes of the highly cross-linked polymer that limited the access of the template. Thus, selecting the appropriate molar ratio of EGDMA to 4-VP was propitious to synthesize the MIP particles with high recognition properties and spherical shapes. So molar ratio of EGDMA to 4-VP of 4:1 was selected as the best ratio for the preparation of MIPs.

FTIR Characterization

Figure 4 shows the FTIR spectra of MIPs and NIPs, which were found to have similar chemical structures. The presence of three significant peaks around 1730 (C=O stretching), 1256 and 1150 cm^{-1} (C—O—C stretching) supported the existence poly(EGDMA) in MIPs and NIPs. The characteristic peaks corresponding to C=N stretching (1601 and 1640 cm^{-1}) and C=C stretching (1455 cm^{-1}) from the pyridine rings can also be observed, suggesting that the functional monomer 4-VP was successfully polymerized in MIPs and NIPs. Moreover, the characteristic peaks of the thiocarbonyl group from the iniferter groups at 1208 cm^{-1} (C=S stretching), as well as at 1421 and 1354 cm^{-1} (CH stretching) in MIPs and NIPs^[27] revealed that iniferter was successfully introduced onto the surface of polymers, which may be very useful for further surface modification to improve recognition performance of polymers. In addition, compared with NIPs, MIPs displayed the characteristic peaks of the amino group from glutathione molecules at 3300 cm^{-1} . Thus, it was concluded that the template molecules were embedded in the MIPs.

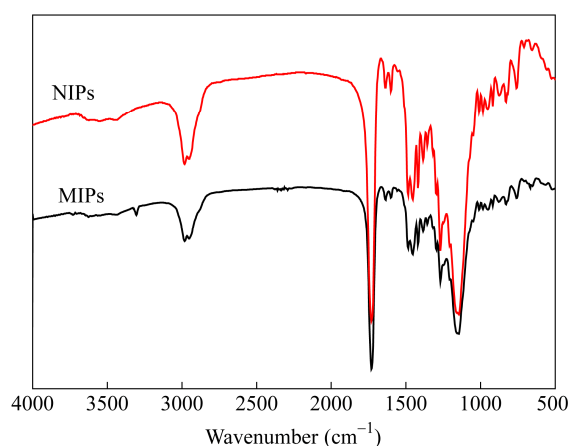


Fig. 4 FTIR spectra of MIPs and NIPs

Adsorption Kinetics

In Fig. 5, the adsorption kinetic curve of the MIPs towards GSH was shown at the GSH concentration of 0.5 $\text{mg}\cdot\text{mL}^{-1}$ in aqueous solution. It could be observed that the adsorption capacity of MIPs towards GSH increased rapidly in initial 150 min, and then slowed down to a plateau. Because the template molecules were polymerized within the MIPs particles, and then the template can be extracted completely following the creation of the imprinted sites during the imprinting step. Also, the template molecules can reach the imprinted sites easily and quickly during the binding step. Therefore, the MIPs exhibited good site accessibility for GSH and the equilibrium was achieved quickly^[36].

In order to further evaluate the adsorption performance of the adsorbents toward GSH, the pseudo-first-order (9), pseudo-second-order (10) and a normalized standard deviation Δq (%) (11) were selected as the following equations:

$$\ln(Q_e - Q_t) = \ln Q_e - k_1 t \quad (9)$$

$$\frac{t}{Q_t} = \frac{1}{k_2 Q_e^2} + \frac{t}{Q_e} \quad (10)$$

$$\Delta q(\%) = \sqrt{\frac{\sum [(q_{e,\text{exp}} - q_{e,\text{cal}})/q_{e,\text{exp}}]^2}{N-1}} \times 100 \quad (11)$$

in which Q_e ($\text{mg} \cdot \text{g}^{-1}$) and Q_t ($\text{mg} \cdot \text{g}^{-1}$) represent the adsorption capacities of MIPs or NIPs towards GSH at time equilibrium and at time t respectively; k_1 and k_2 are the rate constant of the pseudo-first-order and pseudo-second-order respectively; the value of standard deviation is expressed as Δq (%); $Q_{e,\text{exp}}$ and $Q_{e,\text{cal}}$ are the experimental and calculated values of the adsorption capacity respectively; N indicates the number of data points in the experiments.

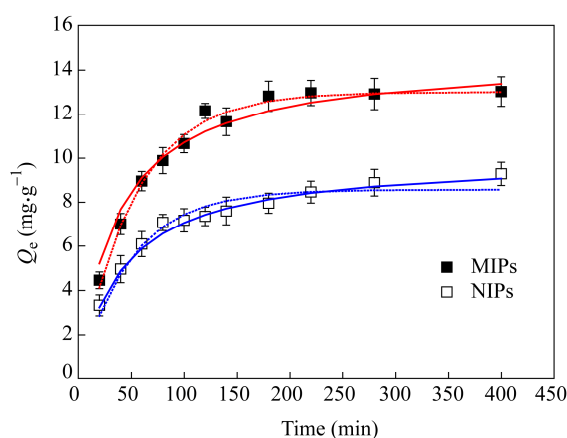


Fig. 5 Pseudo-first-order kinetics (...) and pseudo-second-order kinetics (----) of MIPs and NIPs towards GSH at 25 °C
(Adsorption conditions: amount of MIPs and NIPs: 10 mg; volume: 10 mL; c_{GSH} : 0.5 $\text{mg} \cdot \text{mL}^{-1}$; pH = 7.0.)

Kinetic models for the adsorption of MIPs and NIPs towards GSH are presented in Fig. 5, and the results of kinetic parameters, correlation coefficients (R^2) and standard deviation (Δq (%)) are shown in Table 2. The fit obtained was better with the pseudo-second-order kinetic model than with the pseudo-first-order kinetic model, with the regression coefficient determination having value (R^2) of 0.995 and 0.998 for MIPs and NIPs towards GSH, respectively. Meanwhile, by comparing with the values of standard deviation (Δq (%)), the adsorption of MIPs and NIPs towards GSH were in line with the pseudo-second-order kinetic model. Therefore, the adsorption of MIPs and NIPs towards GSH follows the pseudo-second-order kinetic model, suggesting that the adsorption process is chemical in nature^[37].

Table 2. Kinetic parameters of the pseudo-order for the adsorption of MIPs and NIPs towards GSH at 25 °C

Absorbents	Pseudo-first-order					Pseudo-second-order			
	$Q_{e,\text{exp}}$ ($\text{mg}\cdot\text{g}^{-1}$)	$k_1\times10^{-3}$ (min^{-1})	$Q_{e,\text{cal}}$ ($\text{mg}\cdot\text{g}^{-1}$)	R^2	Δq (%)	$k_2\times10^{-3}$ ($\text{g}\cdot\text{mg}^{-1}\cdot\text{min}^{-1}$)	$Q_{e,\text{cal}}$ ($\text{mg}\cdot\text{g}^{-1}$)	R^2	Δq (%)
MIPs	13.01	18.99	13.02	0.988	9.46	1.903	14.58	0.995	3.54
NIPs	8.56	20.2	9.28	0.942	7.06	2.293	10.18	0.998	5.70

Adsorption Isotherms

Equilibrium binding experiments were then performed to study the rebinding properties of MIPs and NIPs. As shown in Fig. 6, it was observed that the adsorption capacity of GSH on MIPs and NIPs increased rapidly with the increase of GSH concentration from 0 to 1.0 $\text{mg} \cdot \text{mL}^{-1}$. In addition, the adsorption capacity of MIPs for GSH was 13.38 $\text{mg} \cdot \text{g}^{-1}$ higher than that of NIPs (7.62 $\text{mg} \cdot \text{g}^{-1}$). The results indicated that the imprinting process in

MIPs could produce specific imprinting cavities that exhibited high recognition ability for the template. As NIPs lacked the imprinting process, the functional groups were randomly distributed, which resulted in the low adsorption ability of the template.

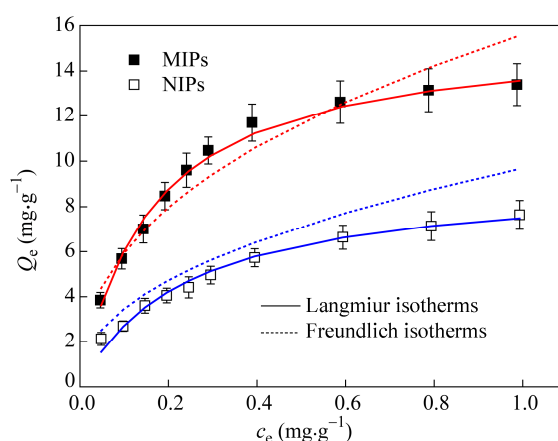


Fig. 6 Adsorption isotherms of MIPs and NIPs towards GSH at 25 °C
(Adsorption conditions: amount of MIPs and NIPs: 10 mg; volume: 10 mL; pH = 7.0; contact time: 400 min.)

To get more insight into the binding parameters of the MIPs, Langmuir and Freundlich models are applied for evaluation of MIPs according to the following equations^[38]:

$$\frac{c_e}{Q_e} = \frac{1}{Q_m K_L} + \frac{c_e}{Q_m} \quad (12)$$

$$\lg Q_e = \frac{\lg c_e}{n} + \lg K_F \quad (13)$$

where Q_e ($\text{mg} \cdot \text{g}^{-1}$) and c_e ($\text{mg} \cdot \text{mL}^{-1}$) are the adsorption capacity and the residual concentrations of GSH in the solution at equilibrium; Q_m ($\text{mg} \cdot \text{g}^{-1}$) is the maximum adsorption capacity of adsorbent at monolayer; K_L ($\text{mL} \cdot \text{mg}^{-1}$) is a characteristic constant more suitable to describe a monolayer adsorption, whereas the Freundlich constant K_F can be used to describe a monolayer adsorption and a multilayer adsorption; n is the Freundlich constant related to temperature.

The adsorption isotherm constants are summarized in Table 3. By comparing the adsorption isotherm of GSH on the adsorbents with Langmuir and Freundlich models, it is evident that the experimental data are more suitable for the Langmuir isotherm rather than the Freundlich model, indicating that Langmuir isotherm with $R^2 > 0.99$ are found to better fit the isotherm data.

Table 3. Adsorption isotherm constants for the adsorption of MIPs and NIPs towards GSH at 25 °C

Adsorbents	Langmuir			Freundlich		
	K_L ($\text{mL} \cdot \text{mg}^{-1}$)	Q_m ($\text{mg} \cdot \text{g}^{-1}$)	R^2	K_F ($\text{mg} \cdot \text{g}^{-1}$)	n	R^2
MIPs	6.49	15.67	0.998	15.65	2.41	0.936
NIPs	4.29	9.24	0.990	9.71	2.26	0.987

Selectivity

The binding selectivity of MIPs and NIPs was also studied by measuring their competitive adsorption capacities toward GSH and its analogs, GSSG and Gly-Gly, whose structures are shown in Fig. 1. Table 4 shows that MIPs exhibited desirable binding selectivity for the template molecule. The adsorption capacities and distribution coefficients of the MIPs for GSH were much higher than those for the structural analogs, thereby verifying the specific recognition toward the template. Moreover, apart from binding GSH, MIPs also adsorbed GSSG and

Gly-Gly, which demonstrated the presence of a certain cross-binding reactivity. Although the same hydrogen bond can form between the structural analogs and functional groups in imprinting cavities owing to the similar structure to the template molecule, the binding amount was much lower than that of the template molecule. Results demonstrated that the specific recognition mechanism of MIPs was related to the interactional force between the template and functional groups, as well as to the complementary match of imprinting cavities with template molecule in size and shape^[37]. The experimental results indeed supported this conclusion, and the selectivity coefficients of MIPs toward GSSG and Gly-Gly were determined to be 2.84 and 4.61, respectively. Compared with MIPs, NIPs had much lower selective coefficients toward the structural analogs due to the lack of imprinted cavities and sites during the polymerization, and the functional sites of 4-VP in the polymer network were arranged in a disorderly fashion. Thus, NIPs was selective toward these compounds through nonspecific interaction^[38].

Table 4. Selectivity coefficients of MIPs and NIPs toward GSH at 25 °C

Adsorbent	MIPs			NIPs			k'
	Q (mg·g ⁻¹)	K_d	k	Q (mg·g ⁻¹)	K_d	k	
GSH	12.32	28.10	—	5.11	10.77	—	—
GSSG	4.71	9.89	2.84	4.53	9.48	1.14	2.49
Gly-Gly	2.96	6.10	4.61	2.15	4.39	2.45	1.88

CONCLUSIONS

A new type of GSH-imprinted polymer particles were successfully synthesized *via* controlled living radical precipitation polymerization at ambient temperature. During the polymerization, the polymerization parameters including the iniferter concentration, monomer loading and molar ratio of cross-linker to functional monomer used not only affected the final particle size but also the specific molecular recognition of the MIPs particles. The static experiments were carried out to evaluate the adsorption kinetics, adsorption isotherms and selective recognition of MIPs particles. It was shown that the MIPs exhibited rapid adsorption kinetics, high adsorption capacity and selectivity coefficients for GSH than NIPs. The adsorption process could be described by pseudo-second order and Langmuir isotherm model. Moreover, the MIP particles also could selectively recognize glutathione over similar analogs, indicating that the MIPs materials could be used as good adsorbents for analysis and determination of GSH.

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