Binding and Recognition Ability of Molecularly Imprinted Polymer Toward *p*-Nitrophenol

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ABSTRACT: *p*-Nitrophenol is a hazardous phenolic compound and its separation and determination is very necessary to protect public health and environmental quality. In this article, macromolecule polyethyleneimine (PEI) grafted on the surface of silica particles was crosslinked using *p*-nitrophenol as a template and diepoxyalkyl (669) as a crosslinking agent, and molecularly imprinted polymer on the surfaces of silica gel MIP-PEI/SiO₂ was prepared. The binding and recognition ability of MIP-PEI/SiO₂ for *p*-nitrophenol were researched using batch and dynamic methods. The experimental results showed that MIP-PEI/SiO₂ had high affinity, specific recognition ability, and excellent selectivity for *p*-nitrophenol. The satu-

rated binding amount could reach to 155.5 mg g⁻¹. The selectivity coefficients relative to *m*-nitrophenol and chlorophenol were 45.80 and 117.5, respectively. The empirical Freundlich isotherm was found to describe well the equilibrium binding data. pH of sample solution was found to have a strong influence on the binding amount. MIP-PEI/SiO₂ was regenerated easily using diluted hydrochloric acid solution as eluent and MIP-PEI/SiO₂ possesses better reusability. © 2012 Wiley Periodicals, Inc. J Appl Polym Sci 125: 2549–2555, 2012

Key words: molecular imprinting; molecular recognition; adsorption; silicas; *p*-nitrophenol

INTRODUCTION

Phenolic compounds have great harmful effect for public health and environmental quality because of its high toxicity and accumulation in the environment. The separation and determination processes of low levels of phenolic compounds are mainly based generally on some available separation or preconcentration material and techniques.

Molecularly imprinting technique is a new method for preparation of highly selective polymer receptors for given molecules. Molecularly imprinted polymers (MIPs) have been used as materials of molecularly recognition in many scientific and technical fields, such as solid-phase extraction,^{1,2} chromatograph separation,^{3–5} membrane separations,⁶ sensors,⁷ drug releases,^{8–10} catalysts,¹¹ etc. The conventional method to prepare MIPs is an entrapment way. The MIPs prepared with conventional methods have some disadvantages. First, the imprinted polymer matrices are usually thick, and the number of recognition sites per unit volume of the polymer is relatively low¹²; second, the template molecules are embedded in the matrices too deeply, so that not only the elution is difficult but also the diffusion barrier for the template molecules is introduced.^{12,13}

The rate of mass transfer is lower, and the template molecules are not easy to bind with recognition sites. To overcome these drawbacks effectively, the surface molecularly imprinted technique has been developed in recent years. The surface imprinted technique can be divided basically into two kinds: (1) the surface imprinted technique based on the emulsion and precipitation polymerization^{13–15}; (2) the surface imprinted technique based on the surface modification of silica gel particles.^{16,17,20,21} For the latter method, there are also several technologies, and among them, grafting polymerization method on the surfaces of silica gel particle is received some attention.¹⁸

Polyethyleneimine (PEI) is a kind of water-soluble polyamine, and there are a great number of amine groups on its macromolecular chains, very strong hydrogen bond interaction can be produced between amine groups of PEI and hydroxyl and nitryl of p-nitrophenol.¹⁹ In this study, functional macromolecule polyethyleneimine (PEI) was grafted onto the surfaces of silica gel particles, and then the molecularly imprinted reaction was carried out toward the macromolecule PEI grafted on the surfaces of silica gel particles using *p*-nitrophenol as a template, diepoxyalkyl (669) as a crosslinking agent. Finally, imprinted polymer MIP-PEI/SiO₂ with high affinity, specific recognition ability and excellent selectivity for *p*-nitrophenol was prepared, and the binding and recognition ability of MIP-PEI/SiO₂ for *p*-nitrophenol were researched using batch and dynamic methods.

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EXPERIMENTAL

Materials and measurements

Silica was purchased from Ocean Chemical Company (about 150 μ m in diameter, pore size: 5 nm, pore volume: 0.8 mL g⁻¹, surface area: 310 m² g⁻¹. Qingdao, China). PEI was purchased from Qianglong Chemical Company (molecular weight, 10,000, Wuhan, China, AR grade). γ -Chloropropyl trimethoxysilane was purchased from Yongchang Chemical Company (Nanking, China, AR grade). Ethylene glycol diglycidyl ether [2,2'-(Ethylenebis(oxymethylene))bisoxirane] (diepoxyalkyl 669) was purchased from Wuxi Wanrong Material Company (Jiangsu, China, CR grade). p-nitrophenol and other chemicals were purchased from Beijing Chemical Plant (AR grade).

Used instruments in this study were as follows: Unic-2602 UV spectrophotometer (Unic Company, American), Perkin-Elmer1700 infrared spectrometer (Perkin-Elmer Company, American), PHS-2 acidimeter (The Second Analytical Instrument Factory of Shanghai, China), THZ-92C constant temperature shaker (Boxun Medical Treatment Equipment Factory of Shanghai, China).

Preparation and characterization of MIP-PEI/SiO₂

The material PEI/SiO₂ was prepared and characterized according to the process described in previous study.^{20,21} The preparation of *p*-nitrophenol-imprinted polymer MIP-PEI/SiO₂ was same as phenol-imprinted polymer MIP-PEI/SiO2²² The process was described as follow. For preparation of MIP-PEI/SiO₂, 1 g of PEI/SiO₂ particles with grafting degree of 5.68% were added into 500 mL of *p*-nitrophenol aqueous solution with initial concentration (C_0) of 1 g L⁻¹. After reaching adsorption equilibrium, 0.3 g of diepoxyalkyl (669) dissolved into 5 mL of absolute ethanol were added into the *p*-nitrophenol aqueous solution. Then, the reaction was allowed to carry out for 6 h at 20°C with continuous stirring. Finally, particles were fully washed with hydrochloric acid solution of 0.01M to remove the template *p*-nitrophenol and the crosslinking agent. After being filtrated and dried, the *p*-nitrophenol-imprinted polymer MIP-PEI/SiO₂ was obtained.

The infrared spectrums of PEI/SiO₂ and MIP-PEI/SiO₂ were determined in order to confirm the chemical structure change of PEI/SiO₂ after molecularly imprinting.

For researching the imprinted effect, the nonimprinted polymer (NIP-PEI/SiO₂) was prepared using the same method and same weight of PEI/SiO₂ and 669 in the absence of the template *p*-nitrophenol.

Measurement of kinetic binding curve and binding isotherm

About 0.1 g of MIP-PEI/SiO₂ was introduced into a conical flask directly. Totally, 50 mL of *p*-nitrophenol

aqueous solution with initial concentration (C_0) of 1 g L⁻¹ and pH of 7 was then added into conical flask. This conical flask was placed in shaker at presettled temperature and oscillated. At different times, the concentration (C_t) of *p*-nitrophenol solution was determined. The binding amount (*Q*) was calculated according to Eq. (1).

$$Q = V(C_0 - C_t)/m \tag{1}$$

where, Q (mg g⁻¹) is the binding amount; V (L) is the volume of the *p*-nitrophenol solution; m (g) is the mass of the absorbent MIP-PEI/SiO₂.

About 0.1 g of MIP-PEI/SiO₂ was introduced into different conical flasks directly. Totally, 50 mL of *p*-nitrophenol aqueous solution with initial concentration (C_0) of 100, 200, 400, 600, 800, 1000 mg L⁻¹ and pH of 7 were then added into each conical flask. These conical flasks were placed in shaker at presettled temperature and oscillated. After binding equilibrium was reached, the equilibrium concentration (C_e) of *p*-nitrophenol solution was determined. The equilibrium binding amount (Q_e) was calculated according to Eq. (2).

$$Q_e = V(C_0 - C_e)/m \tag{2}$$

Influence of pH on binding amount of MIP-PEI/SiO₂

Varying the pH of each sample solution by hydrochloric acid, sodium hydroxide, and buffer solutions (NH_4NO_3 - NH_3 · H_2O and NaAc-HAc), the influence of pH on the binding amount of MIP-PEI/SiO₂ was examined.

Selectivity experiment

To study the selectivity of MIP-PEI/SiO₂ for *p*-nitrophenol, competitive adsorptions of *m*-nitrophenol and chlorophenol with respect to *p*-nitrophenol were also studied by static method. The binary mixed solutions of *p*-nitrophenol/*m*-nitrophenol and *p*-nitrophenol/chlorophenol were prepared. The static binding experiments were performed for the two mixed solutions. After binding equilibrium was reached, the concentrations of *p*-nitrophenol, m-nitrophenol, and chlorophenol in the remaining solutions were determined with spectrophotometry, respectively.

Distribution coefficients of p-nitrophenol, m-nitrophenol, and chlorophenol were calculated by Eq. (3).

$$K_d = Q_e / C_e \tag{3}$$

where, K_d (L g⁻¹) represents the distribution coefficient; Q_e (mg g⁻¹) is the equilibrium binding amount; C_e (mg L⁻¹) is the equilibrium concentration.



Scheme 1 Synthesis process of MIP-PEI/SiO₂.

The selectivity coefficient of MIP-PEI/SiO₂ for *p*nitrophenol with respect to the competitor species (assigned as B) can be obtained from the equilibrium binding data according to Eq. (4)

$$k = K_d(p - \text{nitrophenol})/K_d(B)$$
(4)

where, k is the selectivity coefficient and B represents the *m*-nitrophenol or chlorophenol. The value of k allows an estimation of selectivity of MIP-PEI/SiO₂ for *p*-nitrophenol. A relative selectivity coefficient k'can be defined as expressed in Eq. (5), and the value of k'can indicate the enhanced extent of binding affinity and selectivity of imprinted polymer MIP-PEI/SiO₂ for the template with respect to NIP-PEI/SiO₂.

$$k' = k_{\rm MIP} / k_{\rm NIP} \tag{5}$$

where, k_{MIP} and k_{NIP} is the selectivity coefficient of MIP-PEI/SiO₂ and NIP-PEI/SiO₂ for *p*-nitrophenol, respectively.

Desorption and repeated use experiment

The repeated usability, i.e., regenerability, is an important factor for the absorption material. Desorption of the adsorbed *p*-nitrophenol from the MIP-PEI/SiO₂ also studied by static experimental. Adsorbed *p*-nitrophenol was desorbed with 0.01*M* of hydrochloric acid solution. The MIP-PEI/SiO₂ adsorbed *p*-nitrophenol was placed in the eluent and stirred continuously at room temperature for 2 h. The final *p*-nitrophenol concentration in aqueous phase was determined. Desorption ratio was calculated from the amount of *p*-nitrophenol adsorbed on the MIP-PEI/SiO₂ and final *p*-nitrophenol concentration in the eluent. To test the reusability of

MIP-PEI/SiO₂, *p*-nitrophenol adsorption–desorption procedure was repeated 10 times using the same imprinted polymer.

Dynamics binding and elution experiment

Totally, 1.5 g of MIP-PEI/SiO₂ was filled in a glass column with 8mm of diameter, and the bed volume was 2 mL. The *p*-nitrophenol solution with concentration of 1 g L⁻¹ and pH of 7 was allowed to flow gradually through the column at a rate of five bed volumes per hour (5 BV/h). The effluent with one bed volume was collected, the concentration of *p*-nitrophenol was determined, and the dynamics binding curve was measured. The leaking binding amount and the saturated binding amount were also calculated.

Elution experiment was performed using hydrochloric acid solution with concentration of 0.01M as eluent, and the flow rate was controlled at 1 BV/h. The eluent with one bed volume was collected, the concentration of *p*-nitrophenol was determined, and the elution curve was measured.

RESULTS AND DISCUSSION

Preparing process and structure characterizing of MIP-PEI/SiO $_2$

First, the ring opening reaction between diepoxyalkyl (669) and amine groups of PEI chain takes place when the crosslinking agent diepoxyalkyl (669) is added. Then the template was removed with hydrochloric acid solution. Finally, molecularly imprinted polymer MIP-PEI/SiO₂ was formed. The preparing process of MIP-PEI/SiO₂ was expressed in Scheme 1.

The FTIR spectra of PEI/SiO_2 before and after imprinted were measured and shown in Figure 1.



Figure 1 FTIR spectrum of PEI/SiO₂ and MIP-PEI/SiO₂.

After imprinted, the absorption band at 1191 cm^{-1} is strengthened distinctly, which is the characteristic absorption of C-N bond of tertiary amine group. Whereas all the absorption bands at 3584, 1660, and 802 cm^{-1} , which are vibration absorption of N–H bond, disappear, and the disappearance of these bands reveals that the H atoms of the primary and secondary amine groups in PEI chains have been substituted completely by alkyl, namely, all of primary and secondary amine groups in PEI chains have changed into tertiary amine groups. At the same time, the vibration absorption of hydroxyl has been appeared at 3440 cm⁻¹, and it indicates further that the ring opening reaction has been occurred. The above observations show fully that the crosslinking reaction have been taken place between amine groups of macromolecules PEI and epoxy bond of crosslinking agent 669, and a layer of molecularly imprinted polymer has been formed on the surfaces of silica gel particles, i.e., the surface molecularly imprinted polymer MIP-PEI/SiO₂ has been obtained.



Figure 2 Kinetic binding curve of MIP-PEI/SiO₂ for *p*-nitrophenol. Temperature: 20° C; pH = 7; Initial *p*-nitrophenol concentration: 1000 mg L⁻¹.



Figure 3 Binding isotherms of MIP-PEI/SiO₂ for *p*-nitrophenol at different pH. Temperature: 20°C; Binding time: 8 h.

Kinetic binding curve of MIP-PEI/SiO₂ toward *p*-nitrophenol

The kinetic binding curve was shown in Figure 2. The adsorption of MIP-PEI/SiO₂ toward *p*-nitrophenol reached to equilibrium in 8 h. It was implied that MIP-PEI/SiO₂ possesses high affinity for *p*-nitrophenol. The high affinity attributes to the hydrogen bond interaction and electrostatic interaction between them.

Binding isotherm of MIP-PEI/SiO₂ toward *p*-nitrophenol

The binding isotherms at different pH values were shown in Figure 3. The effect of pH value on the binding amount of MIP-PEI/SiO₂ can be seen clearly from Figure 4, which comes from the data of Figure 3. Obviously, the pH has a great influence on the binding amount of MIP-PEI/SiO₂ for *p*-nitrophenol. In acidic and basic solutions, the binding amounts are all lower, but there is the greatest



Figure 4 Binding amount of MIP-PEI/SiO₂ at different pH.



Figure 5 Plot of LnQ_e vs. LnC_e at different pH.

binding amount in neutral solution. The binding amount's difference at different pH was resulted from different molecule forms of PEI and *p*-nitrophenol and different acting forces between them at different pH. The reasons have been analyzed in detail in the previous study.²³

Freundlich adsorption equation and its logarithms form were follows:

$$Q_e = kC_e^n \tag{6}$$

$$LnQ_e = Lnk + nLnC_e \tag{7}$$

where, *k* and *n* are the empirical constant.

The data in Figure 3 were treated using Freundlich adsorption equation, and the straight line was displayed in Figure 5. Linear regression was performed according to the logarithmic form, and the curve of the LnQ_e vs. LnC_e fit satisfactorily to Freundlich equation. The linear regression coefficient, *n* and *k* at different pH were listed in Table I.

Selectivity

Figure 6 is the binding isotherms of NIP-PEI/SiO₂ and MIP-PEI/SiO₂ toward *p*-nitrophenol, *m*-nitrophenol and chlorophenol, respectively. It can be seen that the binding amount of *p*-nitrophenol and *m*-nitrophenol on NIP-PEI/SiO₂ is equivalent nearly and higher than that of chlorophenol. The reason is that the nitryl in *p*-nitrophenol and *m*-nitrophenol is an electron-withdrawing group, and it makes the hydrogen of hydroxyl easier to form hydrogen bond interaction with tertiary amine groups of PEI.

TABLE ILinear Regression Coefficient, k and n at Different pH

pН	2	5	6	7	9	10
Κ	0.05	1.85	2.50	3.87	2.21	0.07
Ν	0.5475	0.5805	0.5736	0.5694	0.5732	0.6576
R	0.9998	0.9994	0.9991	0.9987	0.9993	0.9984



Figure 6 Binding isotherms of *p*-nitrophenol, *m*-nitrophenol and chlorophenol on NIP-PEI/SiO₂ and MIP-PEI/SiO₂. Temperature: 20° C; Time: 8 h; pH = 7.

After imprinted with *p*-nitrophenol, the binding amount of *p*-nitrophenol on MIP-PEI/SiO₂ does not change, but the binding amount of *m*-nitrophenol and chlorophenol on MIP-PEI/SiO₂ is much lower than that of *p*-nitrophenol. The above facts display fully that MIP-PEI/SiO₂ has high affinity, high recognition ability, and special selectivity for *p*-nitrophenol. Further data will be given in Table II.

Competitive adsorptions of *p*-nitrophenol/*m*-nitrophenol and *p*-nitrophenol/chlorophenol on MIP-PEI/SiO₂ from their mixtures were researched by static method. Table II summarized the data of the distribution coefficients K_d , selectivity coefficients k and relative selectivity coefficients k'.

It can be seen that the selectivity coefficients of MIP-PEI/SiO₂ for *p*-nitrophenol/*m*-nitrophenol and *p*-nitrophenol/chlorophenol increase after imprinted. This suggests that the binding abilities of MIP-PEI/SiO₂ for *p*-nitrophenol are very strong, and far stronger than that for *m*-nitrophenol and chlorophenol. The reason for this is that the cavities imprinted by *p*-nitrophenol are nonmatched to *m*-nitrophenol and chlorophenol and chlorophenol are difficult to produce hydrogen bond interaction with

TABLE II Distribution Coefficient and Selectivity Coefficient Data of MIP-PEI/SiO₂

		MIP-PEI/	NIP-PEI/
Ad	dsorbent	SiO ₂	SiO_2
$K_d (L g^{-1})$	p-Nitrophenol	0.229	0.232
	<i>m</i> -Nitrophenol	0.005	0.199
Selectivity coeff	icient (k)	45.80	1.166
Relative selectiv	ive selectivity coefficient (k') 39.28		.28
$K_d (L g^{-1})$	<i>p</i> -Nitrophenol	0.235	0.246
0	Chlorophenol	0.002	0.096
Selectivity coefficient (k)		117.5	2.563
Relative selectivity coefficient (k')		45	.84

2553

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PEI. This results in the binding amount of *m*-nitrophenol and chlorophenol on MIP-PEI/SiO₂ is very poor. The selectivity coefficients relative to *m*-nitrophenol and chlorophenol are 45.80 and 117.5, respectively. This confirms again that MIP-PEI/SiO₂ has high selectivity toward p-nitrophenol.

Scatchard analysis

The binding parameters of MIPs are mainly estimated by Scatchard analysis.²⁴ The Scatchard analysis is a widely used technique in evaluating the affinities of binding sites taking role in a particular adsorption process. Scatchard equation is described as follows:

$$\frac{Q_e}{C_e} = \frac{Q_{\max} - Q_e}{b} \tag{8}$$

where, $b \pmod{L^{-1}}$ is defined as the equilibrium dissociation constant of the binding sites; Q_{max} (mg g^{-1}) is the apparent maximum binding amount. The data in Figure 3 were plotted according to the Scatchard equation. As shown in Figure 7, there are two distinct sections within the plot, which can be regarded as straight lines. It suggests that there exist two classes of heterogeneous binding interaction (hydrogen bond interaction and electrostatic interaction) in respect to the affinity for *p*-nitrophenol in MIP-PEI/SiO₂. From the slope and intercept of the straight line, the b_1 and the apparent maximum number Q_{max1} of the higher affinity binding sites can be calculated to be 151.52 mg L^{-1} and 143.24 mg g^{-1} , respectively. Similarly, the b_2 and Q_{max2} values of the lower affinity binding sites are 476.19 mg L^{-1} and 260.71 mg g^{-1} , respectively.

Desorption and reusability

Desorption ratios are very high (98.9%). When hydrochloric acid was used as an eluent, the



Figure 7 Scatchard plot analysis of the binding of *p*-nitrophenol to MIP-PEI/SiO₂.



Figure 8 Curve of reusability.

hydrogen bond interaction between *p*-nitrophenol and PEI is disrupted and subsequently *p*-nitrophenol are released into desorption medium. To show the reusability of the MIP-PEI/SiO₂, adsorption– desorption cycle was repeated 10 times by using same imprinted polymer.

Adsorption–desorption cycle of *p*-nitrophenolimprinted polymer MIP-PEI/SiO₂ was shown in Figure 8. The result clearly shows that the MIP-PEI/ SiO₂ could be used repeatedly without loosing significantly binding amount.

Dynamic binding curve and elution curve

The dynamic binding curve of MIP-PEI/SiO₂ for *p*-nitrophenol was shown in Figure 9. It can be found that when *p*-nitrophenol solution passes through the column packed with MIP-PEI/SiO₂ at a flow rate of 5 bed volumes per hour (5 BV/h) upstream, the leaking appears at 88 BV, the leaking binding amount to be calculated is 112.18 mg g⁻¹,



Figure 9 Breakthrough curve of *p*-nitrophenol on MIP-PEI/SiO₂ column. Temperature: 20° C; Initial *p*-nitrophenol concentration: 1000 mg L⁻¹.



Figure 10 Elution curve of *p*-nitrophenol from MIP-PEI/SiO₂. Temperature: 20° C.

and the saturated binding amount is 144.66 mg g^{-1} . Obviously, analogous to the static adsorption result, the dynamic binding capacity is also very high.

The elution curve of MIP-PEI/SiO₂ was shown in Figure 10. Hydrochloric acid solution with a concentration of 0.01M is used as the eluent, and the eluent at a rate of 1 BV/h flows upstream through the column of MIP-PEI/SiO₂ particles on which the adsorption of *p*-nitrophenol has reached to saturation. It can be seen that the shape of desorption curve is cuspate and without tailing, and it shows the fine elution result. The reason is that in strong acidic solution the amino groups on PEI macromolecules are all protonated nearly, and the N atoms have lost the ability of interact with *p*-nitrophenol completely. The calculation results show that within 15 bed volumes, *p*-nitrophenol is eluted from MIP-PEI/SiO₂ column with a desorption ratio of 98.79%. The fact reveals fully that MIP-PEI/SiO₂ has outstanding elution property and MIPPEI/SiO₂ has excellent reusing property.

CONCLUSION

In this article, imprinting toward polyethyleneimine on the surfaces of silica gel particles was carried out successfully, and novel molecularly imprinted material MIP-PEI/SiO₂ was obtained. The imprinted cavities were distributed in thin imprinted polymer layer, there was a smaller barrier for the diffusion of template, and so it was easy and rapid to bind with those recognition sites for the template. MIP-PEI/ SiO_2 possessed high affinity, specific recognition ability and excellent selectivity for *p*-nitrophenol. The selectivity coefficients relative to *m*-nitrophenol and chlorophenol are 45.80 and 117.5, respectively. Additional, MIP-PEI/SiO₂ has excellent reusability.

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