Adsorption Isotherms on Nicotinamide-Imprinted Polymer Stationary Phase

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Abstract

The molecular-imprinted technique is applied for the preparation of a polymer selector by using methacrylic acid as functional monomer, ethyleneglycol dimethacrylate as the cross-linker, 2,2'-azobisisobutyronitrile as the initiator, and nicotinamide as the template. The adsorption isotherms of nicotinamide and nicotinic acid and the competitive adsorption isotherms of nicotinamide and nicotinic acid on the imprinted stationary phase are determined using rectangular pulse frontal analysis and static method. Aqueous solution is used as the mobile phase in frontal analysis. It is found that the adsorption data fit well to both Langmuir and Freundlich isotherm models.

Introduction

The technique of molecular imprinting consists of the self-assembly of a functional monomer and a template molecule in solution followed by the copolymerization of the functional monomer and an excess of an appropriate cross-linking monomer. After dissolution of the small molecule, the resulting network polymer exhibits a significantly higher affinity for the molecule used as the template than for similar molecules, including closely related isomers (1–5). The molecular imprinted polymer has been applied to chiral separation (6,7), solid extraction (8), biomimic sensor (9), and membrane separation (10,11). The most successful noncovalent imprinting systems are based on commodity methacrylic monomers, such as methacrylic acid (MAA) because its carboxyl group is the most commonly hydrogen-bonding and acidic functional group in molecular imprinting, cross-linked with ethyleneglycol dimethacrylate (EDMA).

Although the selectivity is usually high, the imprinted polymers are generally associated with a poor chromatographic efficiency and the elution of broad and asymmetric peaks (12). One of the causes of the poor performance may be the nonspecific binding that comes from incomplete monomer-template association and nonequivalence of the different binding sites. Another reason for such poor performance is slow mass transfer (2). Mass transfer limitations result in peak broadening and asymmetry. A key to improving the performance of imprinted polymers would thus be either to achieve a narrower site distribution or increase the accessibility of the binding sites. The former requires chemical modifications, whereas the latter can be affected by changing the polymer morphology. In order to clearly understand the influence of such modifications, an investigation of the thermodynamics is necessary, which involves the measurement of the adsorption equilibrium isotherms.

In this work, a new imprinted stationary phase was developed using nicotinamide as the template, MAA as the functional monomer, and EDMA as the crosslink. Furthermore, the static and competitive isotherms of nicotinamide and nicotinic acid (based on the imprinted polymer) were determined using rectangular pulse frontal analysis and static method.

Experimental

Materials

Nicotinamide and nicotinic acid were purchased from Fangcao Reagent Company (Beijing, China). Methacrylic acid was from the Tianjing Chemical Reagent Company (Tianjing, China). Ethylene glycol dimethacrylate was purchased from Acros, (Phillipsburg, NJ). 2,2'-Azobisisobutyronitrile (AIBN) was produced by Shanghai Chemical Plant (Shanghai, China) and refined before use. Methanol was from Yili Refined Chemical Co. (Tianjin, China). Chromatographic-grade acetonitrile was purchased from Fisher (Fair Lawn, New Jersey). All other solvents used in the experiment were high-performance liquid chromatography (HPLC) or analytical grade.

Preparation of the stationary phase and packing of the column

The polymer was synthesized following the general imprinting protocol. Nicotinamide (1.0 mmol) and MAA (6.0 mmol) were dissolved in acetonitrile in a glass polymerization test tube; 30 mmol

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EDMA and 0.065 g AIBN were then added into the solution. The test tube was purged with nitrogen for 10 min and sealed under vacuum. Polymerization was reacted in a water bath with the temperature maintained at 60°C for 24 h. The polymerized monolith was ground into particles and filtered by passing through a 60-µm sieve combined with repeated suspensions in water to remove the small particles. The dried particles were packed into a 150- × 4.6-mm stainless steel HPLC column. A solution of methanol–acetic acid (9:1) was then used to remove the template. For comparison, a blank polymer was prepared with the same procedure but in the absence of the template.

Chromatography

The HPLC system consisted of JASCO (Tokyo, Japan) PU-1580 and PU-1586 pumps, a sample loop of 5 mL, and variable wavelength UV-1570 detector. Data processing was carried out with a JASCO LC-1500 workstation. In frontal analysis, the chromatographic conditions were: mobile phase, water; flow rate, 3.0 mL/min; injection volume, 4.0 mL; temperature, 25°C; and UV wavelength, 280 nm in order to determine the steps even at higher concentrations. An ODS column (4.6- × 250-mm) packed with 5-µm particles using methanol–water (50:50) as the mobile phase with a flow rate of 1.0 mL/min and a wavelength of 254 nm were used to determine the free concentration of the compound in the static method. The retention factor (k) was calculated using:

$$(k) = \frac{(t - t_0)}{t_0} \qquad \text{Eq. 1}$$

where t is the retention time of the compound and t_0 is the dead time of the column as determined using $NaNO_2$ as the marker.

Determination of the isotherms

Experimental measurement of breakthrough curves

The static method was performed by placing the sized and washed polymer particles (30 mg) and 3 mL water at different concentrations of the nicotinamide into 10 flasks. The flasks were oscillated in a constant temperature bath at 25°C for 12 h. The mixtures were transferred into individual centrifuge tubes and centrifuged at 4000 rpm for 5 min. The concentration of the free compounds in the solutions were determined using an ODS column and at 254 nm. q was calculated by subtracting the free concentrations from the initial concentrations.

As in the determination of a single component isotherm by the rectangular pulse method, a concentration of the sample was injected and washed off with the mobile phase and then a new higher concentration was injected. The amount of compound accumulated on the stationary phase was calculated using:

$$q = \frac{C(t_R - t_0)}{Ft_0} \qquad \text{Eq. 2}$$

where t_R is the retention time of the breakthrough curve as obtained from half height of the concentration step. The parameter t_0 is the dead time of the column, and F is the phase ratio.

For the determination of the competitive isotherms by the rectangular pulse method (e.g., a binding mixture of nicotinamide and nicotinic acid), two plateaus were obtained in the elution profiles. The first is pure nicotinic acid (the less retained) and the second was composed of nicotinamide (A) and nicotinic acid (B), and their concentrations were the same as that of the sample injected. According to the literature (13,14), the concentration of A and B in the stationary phase can be given by the following expressions, component A:

$$q_A = \frac{(V_2 - V_D)C^A - (V_2 - V_1)(C^A)}{V_{sp}} \qquad \text{Eq. 3}$$

$$q^B = \frac{(V_2 - V_D)C^B}{V_{sp}} \qquad \text{Eq. 4}$$

where q^M and c^M are the concentrations of M (A or B) in the stationary phase and in the mobile phase at equilibrium, respectively; C^A is the concentration of A on the intermediate plateau; V_1 and V_2 are the elution volumes of the two elute plateau; V_D is the dead volume of the column; and V_{sp} is the volume of the stationary phase in the column.

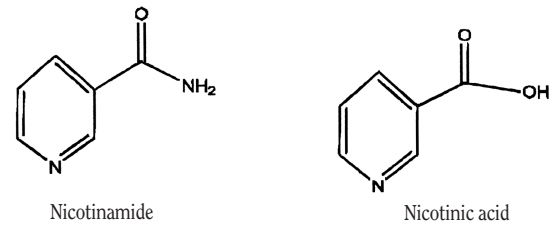
Measurement of the dead time and phase ratio

The dead time was measured by injecting 5 µL $NaNO_2$ into the column. A t_0 value of 0.732 min was obtained. The column was then removed and the injector was connected to the detector directly, and a t_0' of 0.055 min was obtained. By subtracting t_0 to t_0' a value of 0.67 min was obtained. This value was used to calculate the phase ratio F as $F = 0.227$.

Results and Discussion

Selectivity of the imprinted polymer

The structures of the template and its analogues were shown in Figure 1. The retention factors (k) of nicotinamide and nicotinic acid on nicotinamide-imprinted and blank polymers are listed in Table I. The retention factor of nicotinamide is



Nicotinamide Nicotinic acid

Figure 1. The structures of nicotinamide and nicotinic acid.

Table I. Selectivity of the Nicotinamide-Imprinted Polymers

	Retention factor (k)*	
	Nicotinamide	Nicotinic acid
Imprinted polymer	12.27	2.74
Blank polymer	2.89	1.62

* Data was obtained with aqueous mobile phase, flow rate 3.0 mL/min.

higher on the blank polymer than that of its analogue nicotinic acid (see Table I). The reason may be that the template has an amino group and can easily form a hydrogen bond with the monomer. Yet the difference of retention factors is not great, which indicates that the nonspecific binding sites show low selectivity for the compounds. On the other hand, on the imprinted polymer, the retention factor of nicotinamide is more than four times greater than that of nicotinic acid, which indicates the imprinted polymer shows higher affinity and selectivity for the template than its analogue. This is similar to the results found in previous work (15).

Determination of isotherms

The single component isotherms data are shown in Figure 2. The competitive isotherm data of nicotinamide (A) and nicotinic acid (B) are shown in Figure 3; the ratios of the two components were 1:1 and 1:3, respectively. Isotherm data by static method is illustrated in Figure 4.

The Langmuir and Freundich isotherm models were used to fit the obtained data:

$$q = \frac{aC}{1 + bC} \qquad \text{Eq. 5}$$

$$q = aC^{V} \qquad \text{Eq. 6}$$

where C is the total concentration of the injection and *a, b,* and *v* are numerical parameters. The ratio of *a/b* represents the saturation capacities, and *v* is the heterogeneity parameter with a value between 0 and 1.

The imprinted polymer surface is often regarded as heterogeneous, with two kinds of binding sites on the imprinted polymer surface. One is selective or with high-affinity binding energy and the other is nonselective or with low affinity with low binding energy (1,2,14,15). In a low concentration range, the adsorption on selective binding sites is stronger than that on nonselective binding sites (14). In these cases, a bi-Langmuir isotherm model assumes the adsorbent surface to be composed of two different site classes, although the Freundlich isotherm model assumes that the adsorbent surface has no saturation of sites of different binding energies.

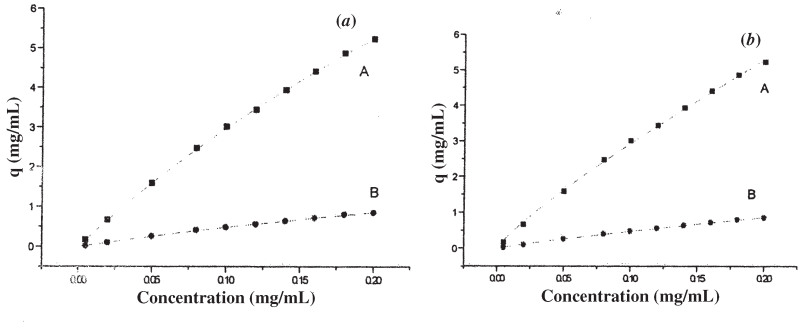


Figure 2. Experimental isotherm data obtained by rectangular frontal analysis and fittings to the Langmuir (*a*) and Freundlich isotherm model (*b*): nicotinamide A and nicotinic acid B.

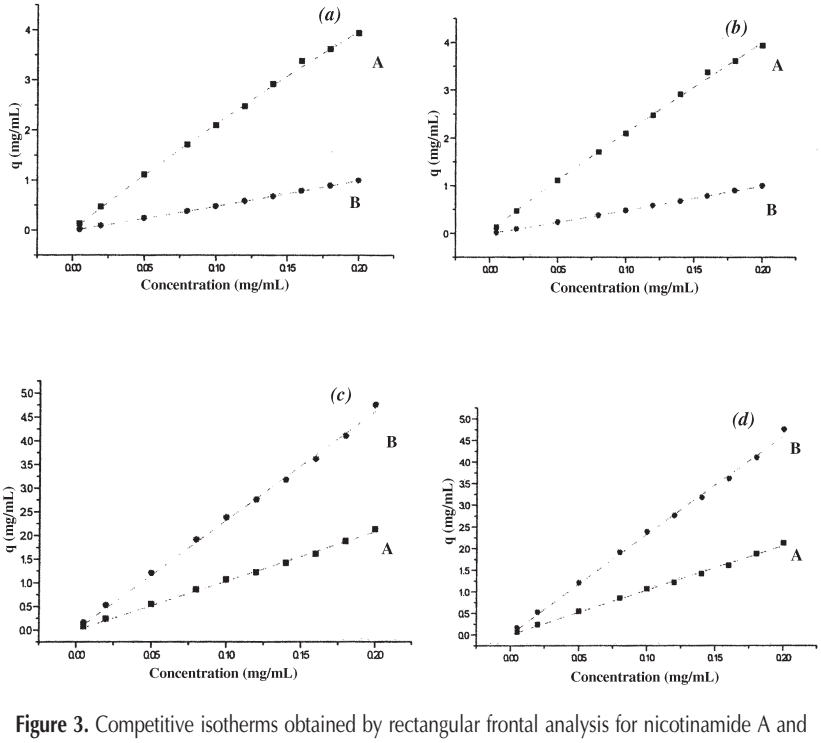


Figure 3. Competitive isotherms obtained by rectangular frontal analysis for nicotinamide A and nicotinic acid B fitted by Langmuir (*a*) and (*c*) and Freundlich (*b*) and (*d*) model. For (*a*) and (*b*): the mass ratio of A to B is 1:1. For (*c*) and (*d*): the mass ratio of A to B is 1:3.

In this work, isotherm data were fitted with both simple Langmuir and Freundlich isotherm models. From the single component isotherm in Figure 2, it can be seen that both the Langmuir and Freundlich isotherms fit well to the experimental data. From the parameters fitted with the Langmuir isotherm model listed in Table II, the binding parameter *a* of nicotinamide is approximately 22 times than that of nicotinic acid, which indicates that the imprinted polymer has much higher selectivity for the template compound than its analogue. This is in agreement with the results obtained by comparison of the retention factors on imprinted and blank polymers. A simple Langmuir isotherm model is ideal and assumes the surface of the particle is homogenous. All of these indicate that the polymer surface shows higher homogeneity, and that the nonspecific binding sites adsorption is small in the tested concentration.

On the other hand, from Figures 2 and 3, it is apparent that the fitting plot of data to a simple Langumir isotherm is nearly linear. This shows that the adsorption isotherm is linear in the tested concentration range. At higher concentrations, because the UV absorbance of the tested compounds will be outside the linear range, the isotherm data illustrated in Figure 4 was obtained by the static method. Though the concentration range is approximately 15 times larger than that shown in Figures 2 and 3, obvious curvature still cannot be obtained. The unbound fractions of

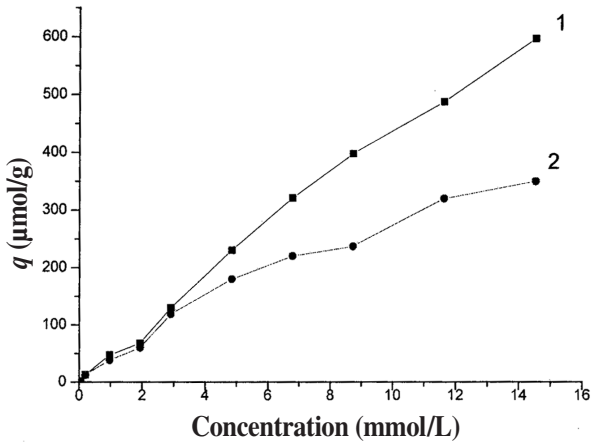


Figure 4. Experimental isotherm data obtained by static method: nicotinamide-molecular imprinted polymer (1); blank-molecular imprinted polymer (2).

Table II. Single Component Isotherm Parameters Fitted by Both Isotherm Models

	Langmuir		Freundlich	
	a	*b*	*a*	*v*
Nicotinamide	34.480	1.574	20.896	0.852
Nicotinic acid	5.648	1.590	3.414	0.851

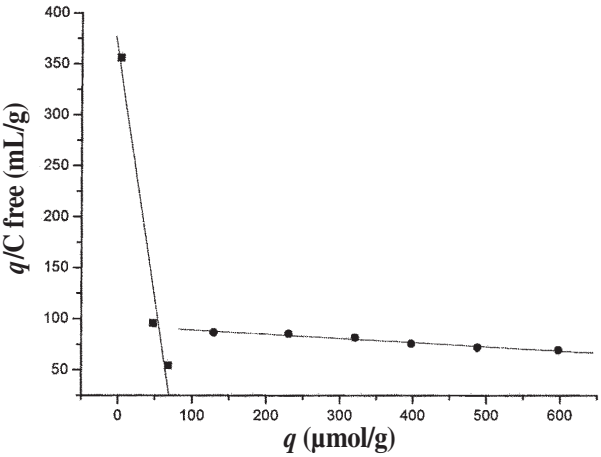


Figure 5. Scatchard plot of nicotinamide imprinted polymer for nicotinamide.

Table III. Competitive Isotherm Parameters of Nicotinamide and Nicotinic Acid Fitted by Both Isotherm Models*

	Nicotinamide				Nicotinic acid			
	Langmuir		Freundlich		Langmuir		Freundlich	
CA:CB	a	*b*	*a*	*v*	a	*b*	*a*	*v*
1:1	22.905	0.750	17.612	0.920	4.654	–0.357	5.378	1.045
1:3	10.385	–0.0167	10.113	0.985	23.325	0.0352	22.378	0.981

* The data were obtained by frontal analysis.

nicotinamide (C_{free}/C_{total}) are almost constant, and a Scatchard plot (Figure 5) showed that there were two kinds of adsorption sites. It was different from the result of the Langumir isotherm. The template has two types of sites to interact with MAA. More work is needed to explain the reason.

The competitive isotherm results of nicotinamide and nicotinic acid are listed in Table III and Figure 3. It can be seen that when the mass ratio of nicotinamide and nicotinic acid is 1:1, the binding parameter of nicotinamide is more than three times greater than that of nicotinic acid. This is nearly the same as those of single component isotherms, which indicates that the competitive effect is not significant. Similar results are also be obtained when the mass ratio of nicotinamide and nicotinic acid is 1:3.

Conclusion

From the mentioned results, conclusions can be drawn that the imprinted polymer shows a high degree of homogeneity. The imprinted polymer exhibits higher affinity for the template because it shows a higher binding constant than its analogue. The potential high saturation capacity and higher selectivity of the developed polymer is important for the preparation of drugs with high purity. The polymer was also stable and can be reproducibly synthesized, which is an attractive feature for further applications. This work also shows that frontal analysis is an easy and accurate method for the determination of adsorption isotherm data in molecular-imprinted polymers. For determining the adsorption isotherm, the equilibrium concentrations of bound and free template has to be reliably measured within a large concentration interval, but in frontal analysis, the tested concentration range was not always large enough because of the compound's limited solubility and high UV absorbannce. This, to some degree, forbade the determination of accurate values of the isotherm coefficients.

Acknowledgments

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