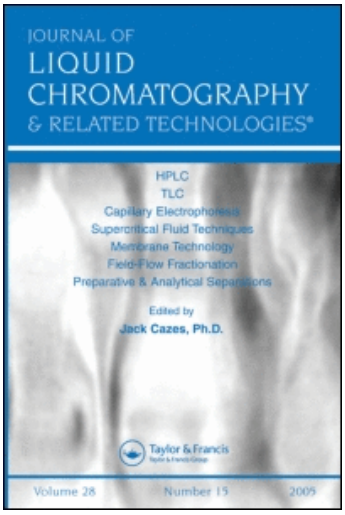

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Molecular Recognition Properties and Adsorption Isotherms of Diniconazole-Imprinted Polymers

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Abstract: Diniconazole, a triazole-type fungicide with a broad antifungal spectrum, shows excellent efficacy against various diseases. Molecular imprinted polymers of the compound were prepared by the technique of non-covalent molecular imprinting polymerization. The functional monomers used include methacrylic acid, acrylamide, or a combination of methacrylic acid and acrylamide. HPLC was used to study the molecular recognition mechanism regulating the binding behavior and evaluate the binding performance of MIPs for template and for paclobutrazol. The result showed that the MIPs (P1-3) had significant molecular imprinting effect, but P3 is better for the separation of diniconazole and paclobutrazol. The adsorption isotherms of diniconazole on the imprinting stationary phase were determined using rectangular pulse frontal analysis. Three different ratios of methanol-water (50 : 50, 55 : 45, 60 : 40, v/v) were used as the mobile phases in frontal analysis. It was found that the bigger the ratio of methanol is, the better adsorption lines are fitted to both Langmuir and Freundlich isotherm models.

Keywords: Molecular imprinting, Diniconazole, Molecular recognition, Adsorption isotherms

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INTRODUCTION

The molecular imprinting technique is an effective strategy to prepare stationary phases having a specific molecular recognition.[1–3] The principle of the molecular imprinting is that a target molecule (template) and functional monomers are polymerized with a crossing reagent. After removal of the template, the functional groups in the resulting binding sites should be arranged in positions suitable for interaction with the template molecule; the molecule imprinting polymers (MIPs) can selectively recognize the template molecular among other structurally related molecules. MIPs are resistant not only to mechanical stress, high pressure, and elevated temperature, but also to acids, bases, organic solvents, and metal ions. One way to obtain the MIPs is the production of bulk polymers, which have to be ground and sieved. This process is well established, especially when the polymer is to be utilized in columns for HPLC or for assays. However, the procedure is time consuming and, unfortunately, implicates a loss of material due to the need of removing fine particles from the usable remains. A polymer yield of useful particles is typically around 20%. To avoid that disadvantage an alternative method was found, namely the generation of microbeads. These materials can be used directly after production, but the template has to be removed via extraction. It is even more advantageous, because these microbeads can be produced in a uniform manner.

All chromatography responses depend primarily on the equilibrium isotherms of the components of the mixture between the two phases of the chromatographic system. A better understanding of the chromatographic separation process, as well as the ability to predict the performance of a chromatographic procedure, has become necessary for the development of preparative application chromatography, which is acquiring a great deal of importance in the pharmaceutical industry and in life science.

Nowadays, society is highly sensitive towards contaminants in foodstuff. Such substances may be pesticide, herbicides, hormones, and antibiotics. It has been demonstrated that MIPs are applicable for the determination of food additives, such as peptides[4] and flavor additives. In most cases, MIPs for peptide derivatives have been prepared in organic media and have been utilized for separations in organic mobile phases by HPLC.[5–7] Recently, the MIPs prepared in organic media have been subsequently used in aqueous environments.[8]

Diniconazole is a triazole-type fungicide with a broad antifungal spectrum. This compound shows excellent efficacy against various diseases on cereal, fruits, and other fields of crops by both preventive and curative applications.[9] It is a specific inhibitor of the 14α-demethylation step of ergostem biosynthesis in fungi.[10]

In this paper, we prepared MIPs for diniconazole and evaluated the recognition ability in hydro-organic mobile phase. We observed an abnormal phenomenon, because when increasing the ratio of water in the mobile

phase, the k value became larger. So, adsorption isotherms of diniconazole on the imprinted polymer in different mobile phases were determined using rectangular pulse frontal analysis. Based on the results obtained, the retention and separation mechanisms of diniconazole are discussed.

EXPERIMENTAL

Chemicals

Diniconazole and paclobutrazol were purchased from Factory of Limin (Yancheng, China), ethylene glycol dimethacrylate (EDMA) from Acros (New Jersey, USA), methacrylic acid (MAA) from Tianjing Chemical Reagent Company (Tianjing, China), acrylamide (AM) and methanol from Yili Refined Chemical Co. (Beijing, China), 2,2′-azobisisobutyronitrile (AIBN) from Shanghai Chemical Plant (Shanghai, China) and refined before used.

Chromatography

The chromatography system consisted of JASCO (JASCO, Japan) PU-1587 pumps, a variable wavelength UV-1570 detector; date processing was carried out with a JASCO LC-1500 workstation, flow rate was 1.0 mL min^{-1}. When the retention and separation mechanisms were studied, the mobile phase was composed of methanol and water (48 : 52,v/v), UV wavelength was set at 230 nm. In frontal analysis, three mobile phases were used: the ratio (v/v) of methanol and water were 60 : 40, 55 : 45, 50 : 50, respectively, injection volume 4 mL, UV wavelength was set at 290 nm in order to determination the steps even at higher concentration. The column was ODS column (JASCO, 250 × 4.6 mm).

Preparation of Molecular Imprinted Polymers

Diniconazole (1 mmol) and MAA (4 mmol) were dissolved in 40 mL of toluene in a glass polymerization test tube, then EDMA (20 mmol) and AIBN (50 mg) were added into the solution. The test tube was purged with nitrogen for 10 min and sealed under vacuum. After that, polymerization was reacted in a water bath with the temperature maintained at 55°C for 24 h. Other MIPs were prepared in a similar manner using acrylamide (AM) or the mixture of MAA and AM as the monomer. The dried microbeads polymers were packed into a stainless-steel column (4.6 mm I.D × 50 mm). Then methanol was used to remove the template. For comparison, blank polymer (BP) was prepared with the same procedure but in the absence of the template.

Determination of the Isotherms

When isotherms are determined by the rectangular 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:

$$Q = C(t_R - t_0)/Ft_0 \quad (1)$$

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

Measurement of the Hold-Up Time and Phase Ratio

The hold-up time was measured by injecting 2 μL acetone into the column. A t_0 value of 1.005 min was obtained. Then the column was removed and the injector was connected to the detector directly and a t_0' of 0.174 min was obtained. By subtracting t_0 to t_0', a value of 0.831 min was obtained. This value was used to calculate the phase ratio F and $F = 0.427$.

RESULTS AND DISCUSSION

Selectivity of Functional Monomers

In non-covalent imprinting, methacrylic acid (MAA) is commonly used as a functional monomer. This reagent can act as a hydrogen bond donor or acceptor and has an acidic proton, enabling ionic interactions to be utilized in the imprinting process. From observations, we found that when MAA was used as functional monomer, the capacity factor of diniconazole on P1 is much larger than on BP1 for the same mobile phase, this indicates that P1 has a molecular imprinting effect for diniconazole, but the non-specific adsorption is too strong, as could be seen from Table 1. Moreover, when

Table 1. The effects of difference functional monomers on capacity factor

Polymer capacity	Template factor (k)	Functional monomer	Mobile phase (methanol : water)
P1	Diniconazole	MAA	44 : 56
BP1 47.7	None	MAA	44 : 56
P2 17.1	Diniconazole	AM	40 : 60
BP2 0.6	None	AM	40 : 60
P3 20.7	Diniconazole	MAA + AM	48 : 52
BP3 1.1	None	MAA + AM	48 : 52

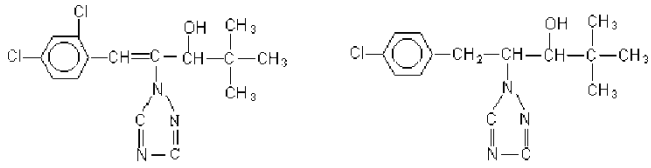

Figure 1. Chemical structural of diniconazole and paclobutrazol.

the ratio of water was increased the capacity factor became bigger; when only water was used as mobile phase, diniconazole could not be eluted entirely both on P1 and BP1. The retention of diniconazole on BP1 is mainly because of hydrophobic interaction since 2,4-dichlorophenyl is a hydrophobic group. The same phenomenon was also observed on other columns discussed below. When AM was used as a functional monomer, diniconazole was imprinted on polymer, apparently because it was retained strongly on P2 but eluted quickly from BP2. The selectivity test of MIPs was carried out using paclobutrazol, which was selected either for its structural interest or its purpose of use. The chemical structures of diniconazole and paclobutrazol are shown in Figure 1. When the composition of the mobile phase is methanol and water (50 : 50,v/v), which is the optimum mobile phase, the resolution of diniconazole and paclobutrazol could reach 1.4 on P2, but baseline separation could be achieved between diniconazole and paclobutrazol on P3, which was shown in Figure 2. This suggests that MAA and AM have some interactions

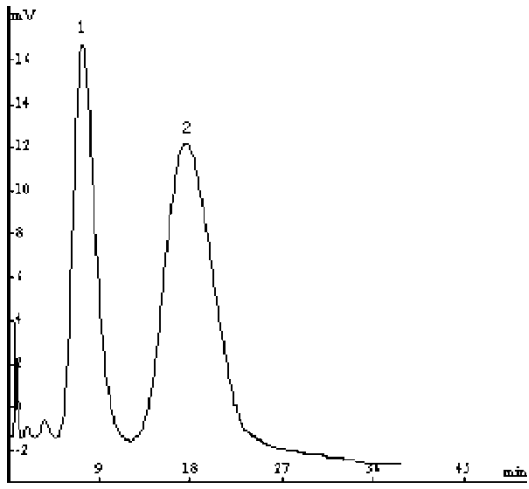

Figure 2. Chromatograms obtained from a mixture of diniconazole and paclobutrazol on P3.

that increase the interaction between diniconazole and P3. Therefore, P3 was used for further experiments.

Optimizing the Mobile Phase

In the pre-polymerization mixture, the template molecule can interact with the monomers via hydrogen bonding, ionic, π-π, and hydrophobic interactions. Hydrogen bonding and ionic forces are typically dominant. The effectiveness of these interactions is highly dependent upon the polarity of the medium, thus organic solvents of low polarity are used to obtain optimal imprints. Through observation, we found that diniconazole eluted quickly when methanol, acetonitrile, isopropanol, or chloroform was used as mobile phase. When only water was used as mobile phase, diniconazole could not been eluted entirely. This indicates that besides the molecular shape recognition, hydrophobic interactions are the dominating interactions between diniconazole and MIP, so, if we change the ratio of methanol and water, the resolution time of diniconazole is changed accordingly. Figure 3 shows the effect of methanol content on the retention of diniconazole on the P3 column. This suggested that the polymer could be effectively used in a pre-separation or pre-concentration system, such as solid extraction, for the separation of diniconazole from other analogues.

Determination of Isotherms

A relationship between the amount of compound accumulated on the stationary phase (q) and the total concentration of the injection (C) is needed for the

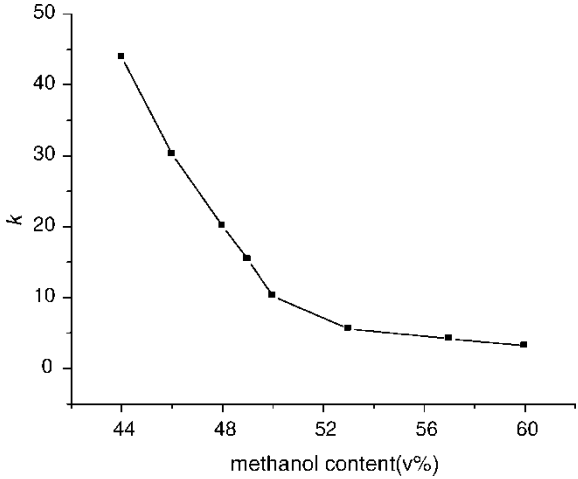

Figure 3. Effect of methanol content on the retention of diniconazole on P3 column.

mass balance to be solved. In this work, two isotherm models were used, the Langmuir and Freundlich idotherm models. The respective equations for these models are written as follows:

$$q = aC/1 + bC \tag{2}$$

$$q = aC^{\nu} \tag{3}$$

In these equations, a, b, ν are numerical parameters. The ratio of a/b represents the saturation capacities of the compound studied on the corresponding

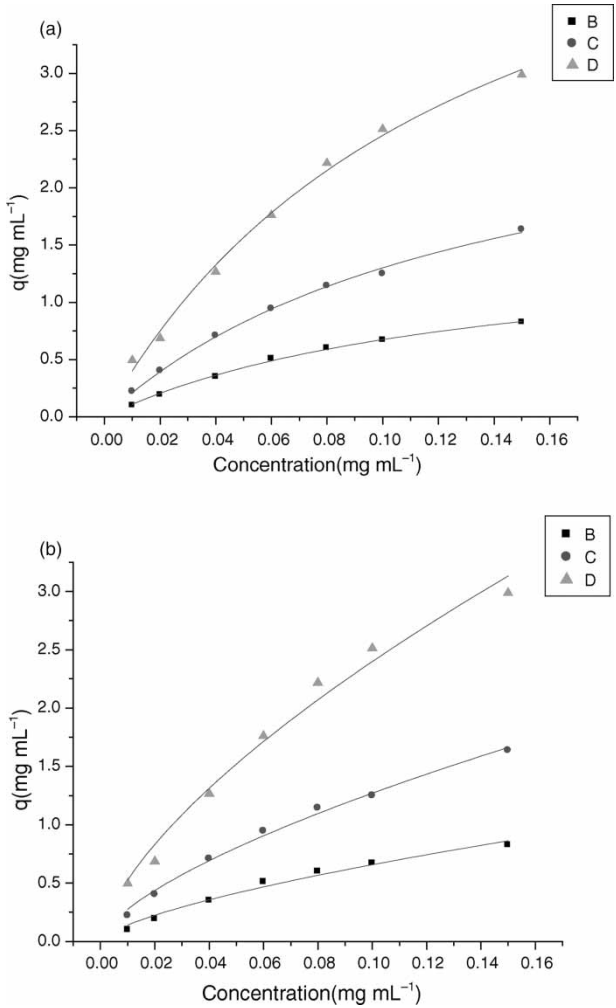

Figure 4. Experimental isotherm data (symbols) obtained by rectangular frontal analysis and fittings (lines) to the Langmuir (a) and Freundlich isotherm (b) model. B = 60 : 40, C = 55 : 45, D = 50 : 50 (methanol : water, v/v).

Table 2. Isotherm parameters fitted by both Langmuir and Freundlich isotherm models

	Langmuir		Freundlich	
	a	b	a	ν
B	11.833	7.547	3.059	0.667
C	22.632	7.391	5.845	0.662
D	43.223	7.584	10.906	0.658

B, C, D represent the ratio of methanol and water in the mobile phase, B = 60 : 40, C = 55 : 55, D = 50 : 50 (v/v).

adsorption sites of the stationary phase. ν is the heterogeneity parameter between 0 and 1.

Figure 4 is the isotherm fitted both by Langmuir and Freundlich isotherm models. It can be seen that when increasing the ratio of methanol in the mobile phase, two isotherm models better fit the experimental data. From the parameters fitted by two model in Table 2, the saturation capacities (a/b) of the selective sites increased (from 1.568 to 5.699) when the ratio of water increased (from 40% to 50%) in the mobile phase, at the same time the heterogeneity factor decrease from 0.667 to 0.658. It seems that increasing the ratio of water in the mobile phase activates new sites and that, in the same time, the adsorption energy distribution broadens.

CONCLUSION

Three different diciconazole imprinted polymers P1, P2, and P3 were prepared using methcrylic acid, acrylamide, or a combination of methacrylic acid and acrylamide, respectively, as the functional monomers. Significant molecular imprinting effects were observed for three kinds of MIPs, but P3 is the most ideal one. Frontal analysis is an easy and accurate method for determination of adsorption isotherm data in molecular imprinted polymers. The results showed that the saturation capacities of the selective sites increased when the ratio of water in the mobile phase increased. At the same time the heterogeneity factor drops, which indicated that the hydrophobic interaction is dominating interactions between diniconazole and MIP. Because the more content of water in the mobile phase, the longer retention time was obtained, it shows that diniconazole-imprinted polymer has potential application in the enrichment, separation, and detection of diniconazole in biological fluids.

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