Chinese Journal of Chemistry, **2004**, *22*, 275—278 **Article**

Molecularly Imprinted Polymer with Calix[4]arene Derivative for the Recognition of Acetanilide

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Two molecularly imprinted polymers binding to analgesic acetanilide were prepared using either dual functional monomers of calix[4]arene derivative and acrylamide or single monomer acrylamide, respectively. The polymers were ground, sieved and investigated by equilibrium binding experiment to evaluate their recognition properties for the template and other substrates. Scatchard analysis showed that homogeneous recognition sites were formed in the imprinted polymer matrix. Our results demonstrated that the polymer using two functional monomers exhibited better selectivity for the template. This study may open new frontiers for the development and application of imprinted polymers, such as drug separation and purification.

Keywords molecularly imprinted polymer (MIP), functional monomer, calixarene, acetanilide

Introduction

Molecular imprinting is a novel technique for constructing tailor-made receptor binding sites in a synthetic polymer matrix.[1] In this method, an objective substrate is employed as a template molecule that binds functional monomer during the copolymerization of the monomer and crosslinking agent. Subsequent removal of the template leaves behind cavities on the polymer matrix with a shape and an arrangement of functional groups resulting in complementary binding sites to the original template. These sites enable the polymer to rebind the template molecules selectively. Therefore it is evident that the functional monomers are crucial to the specificity of the molecularly imprinted polymers (MIPs).

The most common functional monomer is methacrylic acid (MAA). Others such as acrylic acid, 4-vinylpyridine, 2-(trifluoromethyl) acrylic acid[2] and 2-acrylamido-2-methyl-1-propane sulfonic acid,[3] are also well known. Recent progress has been achieved to explore various categories of functional monomers to make molecular imprinting more versatile as a tailor-made receptor synthesis method. A number of compounds have been chosen as the template including drug species, amino acids and peptides, nucleotide bases, sugars, steroids and herbicides.[4,5] It is reported that MIPs prepared by β-cyclodextrin (β-CyD) as new functional monomer can efficiently recognize cholesterol in aqueous media.[6-8] The Takeuchi's work group synthesized MIPs as 9-ethyladenine receptor using a metalloporphyrin-based functional monomer.[9,10] Furthermore, two functional monomers have been used together to enhance separation and recognition properties. Nichllos and Piletsky combined Bisacryloyl β-CyD and 2-acryloylamido-2,2'-dimethylpropane sulfuric acid for the separation of *D*- and *L*-phenylalanine.[11]

Similar to β-CyD, calixarenes also contain cavities of sufficient diameter and depth to form host-guest complexes with a wide variety of compounds, and have been well studied due to their peculiar molecular recognition ability. However, calixarenes have not yet been reported for use in molecular imprinting. In this paper we first attempt to apply two different functional monomer species of calixarenes derivative **1** (25,27-diallyl-26,28-dihydroxycalix[4]arene) (Figure 1) and acrylamide to molecular imprinting with acetanilide as template molecule. Acetanilide is one of the analgesics used in clinic to relieve pain and reduce fever, and its structure is suitable to form host-guest complex with **1**.

Experimental

Materials and instruments

Acetanilide was purchased from Kermel Chemical Reagent Development Center (Tianjin), and was recrystallized after being dissolved in hot water. Aniline was

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Received July 7, 2003; revised and accepted December 20, 2003.
Project supported by the National Natural Science Foundation of China (Nos. 20175009 and 20375017), the Doctoral Foundation of Education Ministry of China (No. 2002055002) and Tianjin Natural Science Foundation (No. 033603511).

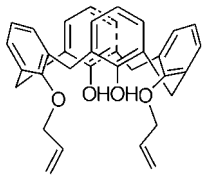

Figure 1 Structure of 25,27-diallyl-26,28-dihydroxycalix-[4]arene (**1**).

purchased from Tianjin Chemical Reagent No. 1 Factory, acetaminophen from the National Institute for the Control of Pharmaceutical and Biological Products (China). Ethylene glycol dimethacrylate (EGDMA) was synthesized by Anli Chemical Co., Ltd. (Suzhou City), acrylamide (AA) by Tianjin Chemical Reagent Research Institute, 2,2-azobisisobutyronitrile (AIBN) by Nankai University Special Reagent Factory. All other chemicals were of analytical grade.

A Shimadzu UV-240 double-beam spectrophotometer and a SHZ-82 constant temperature bath oscillator were used.

Molecular imprinting

Calixarenes derivative **1** was prepared as described in Ref. 12.

Preparation of P(I): Acetanilide (template, 67.58 mg, 0.5 mmol) was dissolved by dichloromethane (3 mL) in a 25-mL thick wall glass test tube before calixarene derivative **1** (252.3 mg, 0.5 mmol), AA (142.16 mg, 2 mmol), EGDMA (cross-linking agent, 1.982 g, 10 mmol) and AIBN (initiator, 50.0 mg) were added under sonication. After nitrogen gas bubbled into the solution for 5 min, the test tube was sealed under vacuum and then kept in a water-bath at 60 ℃ for 24 h. The resulting bulk rigid polymers were crushed to pass through 75 μ sieve. The particulates were extensively washed with methanol-acetic acid (9/1, *V*/*V*) until no template was detected in the washing solution by spectrophotometer. Finally the particulates were washed with methanol to remove residual acetic acid and dried to constant weight under vacuum at 55 ℃. Non-imprinted polymer P(0I) was prepared using the same procedure without the addition of the template. P(II) and P(0II) were prepared only with AA as the functional monomer. All the imprinted and non-imprinted polymers are shown in Table 1.

Binding experiments

The polymer (20.0 mg) was added into a 2 mL ethanol solution of 2 mmol/L template or other substrates in a 25 mL conical flask. The flask was oscillated in a constant temperature oscillator at 25 ℃ for 12 h. Then the mixture was filtrated and the concentration of the filtrate was determined by a spectrophotometer at appropriate wavelength (acetanilide: 242 nm; aniline: 239 nm; acetaminophen: 250 nm). Binding experiments were carried out at various template concentrations range from 0.1 mmol/L to 5.0 mmol/L. The amounts of template or other substrates bound to the polymer Q (μmol) was calculated by the following equation: $Q=(c_0-c_t)V$, where c_0 and c_t are the template or selected substrate concentrations (mmol/L) in the solution which were measured at initial and after filtration respectively. V is the initial solution volume (2 mL).

Results and discussion

Polymer preparation

Calixarenes possess the molecular architecture appropriate for the inclusion of many kinds of organic compounds and ions within the hydrophobic cavity. *p-tert*-butylcalix[4]arene has a more stable and simple conformation than *p-tert*-butylcalix[6]arene and *p-tert*-butylcalix[8]arene. Thus we chose the *p-tert*-butylcalix[4]arene derivative **1** as a functional monomer. The polymer was synthesized by the copolymerization of **1** and EGDMA by the initiation of AIBN at the presence of acetanilide as the template molecule. The interaction between template and functional monomer was illuminated in Figure 2. The phenyl moiety of acetanilide may go into the hydrophobic cavity of **1**, and the acid amide group of acetanilide may interact with the acid amide group of AA to form hydrogen bond. The dual functional monomers created better interactions to increase the selective recognition properties of acetanilide-imprinted polymers.

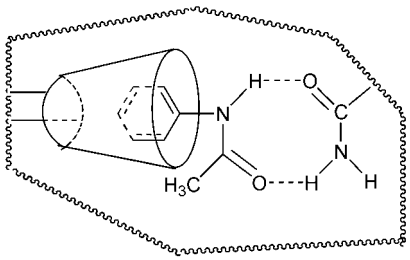

Figure 2 Schematic illustration of the binding of acetanilide in P(I).

Table 1 Polymer compositions

Polymer	Acetanilide/mmol	AA/mmol	Calix[4]arene derivaltive **1**/mmol	EGDMA/mmol	AIBN/mg	CH_2Cl_2/mL
P(I)	0.5	2	0.5	10	50	3
P(0I)		2	0.5	10	50	3
P(II)	1	4		20	50	6
P(0II)		4		20	50	6

Substrate-selectivity of acetanilide-imprinted polymers

Acetanilide and two other compounds with similar structures, aniline and acetaminophen, were used to examine the selectivity of the acetanilide-imprinted polymers. The amounts of substrates bound to the polymers were determined by the equilibrium binding experiment (see Figure 3).

The polymer P(I) imprinted with acetanilide exhibited higher binding capacity as measured by *Q* value for template acetanilide than for aniline and acetaminophen (Figure 3a). The template can not only form hydrogen bonds with the functional monomer of AA but also can go into the cavity of calix[4]arene **1**. Thus the imprinted polymer P(I) with dual functional monomers exhibited a better imprinting effect than P(II). The *Q* value of P(0I) for template acetanilide was much lower than that of P(I) and the selective separation effect was obvious. P(0I) did not have the recognition sites complementary in both shape and functional groups to the template, so that functional groups were distributed randomly throughout the polymer matrix, leading to weak binding sites. Meanwhile, both P(I) and P(0I) can adsorb aniline at a substantial level. An explanation for this observation lies in aniline's structure, which is much smaller than the templates, making it easier to go into the cavity of calix[4]arene **1**. The difference in *Q* values for aniline between P(I) and P(0I) was not obvious, indicating that the binding for aniline was non-specific. On the other hand, P(I) showed extremely poor imprinting behavior to acetaminophen. The only structural difference of acetaminophen and acetanilide was that there was an —OH in acetaminophen (Figure 3). The hydrophobic interactions did not allow the hydrophilic —OH of acetaminophen to go into the hydrophobic cavity of calix[4]arene **1**. This revealed that the two functional monomers of calix[4]arene **1** and AA cooperated to recognize substrates and improved the selective recognition of acetanilide-imprinted polymer.

From Figure 3b we can see that P(II) showed much lower *Q* value for acetanilide compared to P(I), though P(II) had the selective recognition properties for substrates at some extent. The amount of aniline bound to P(II) was much lower than that of P(I), which demonstrated that the functional monomer calix[4]arene **1** of P(I) played an important role in the separation and recognition progress. P(II) exhibited non-imprinting behavior to acetaminophen as P(I). From Figure 3, we also can see that P(II) showed better separation ability than P(I) as for the mixture of acetanilide and aniline. While P(I) can separate the mixture of acetanilide and acetaminophen effectively.

Binding characteristics of P(I)

In an attempt to investigate the affinity of the imprinted polymer for acetanilide, binding experiments and subsequent Scatchard analysis were carried out. We only discuss the binding characteristics of P(I) because P(I) showed better imprinting effect for template than P(II) did. The binding isotherm of acetanilide to the

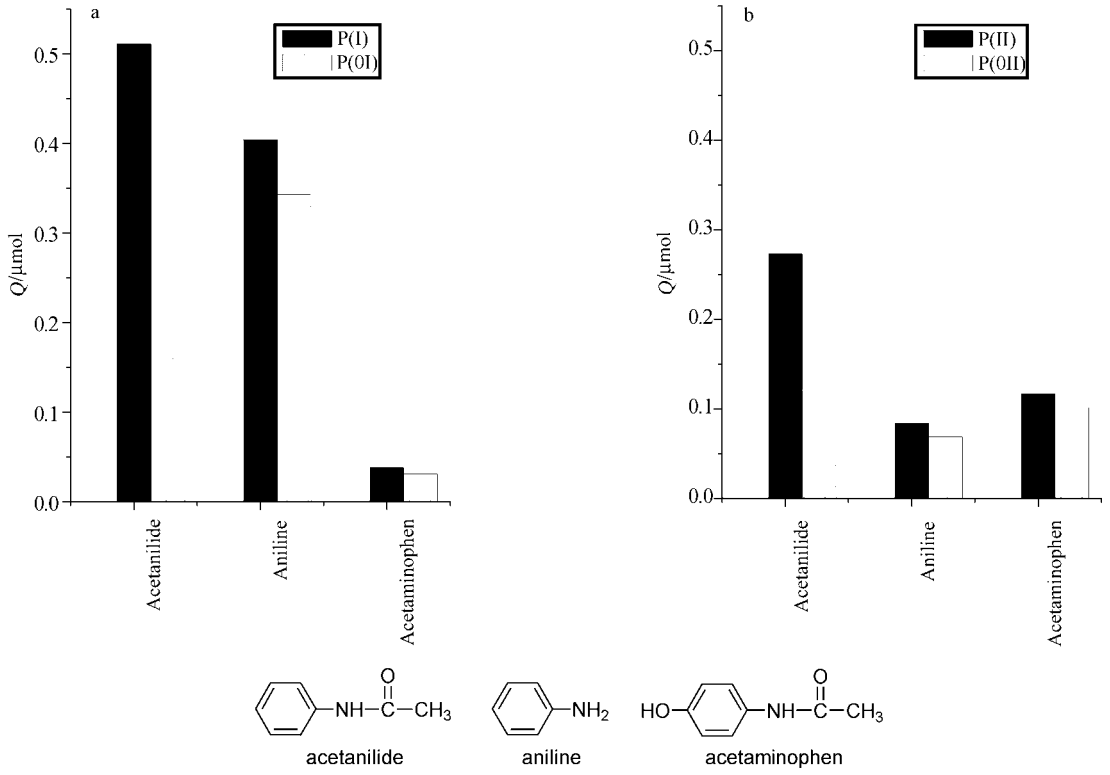

Figure 3 Selectivity of P(I) and P(0I) (a), P(II) and P(0II) (b) to other substrates. *Q*: amount of substrates bound to 20.0 mg polymers; *V* =2.0 mL; adsorption time: 12 h.

imprinted polymer P(II) was determined in the range of 0.1—5.0 mmol/L acetanilide ethanol solution (initial concentration) as shown in Figure 4.

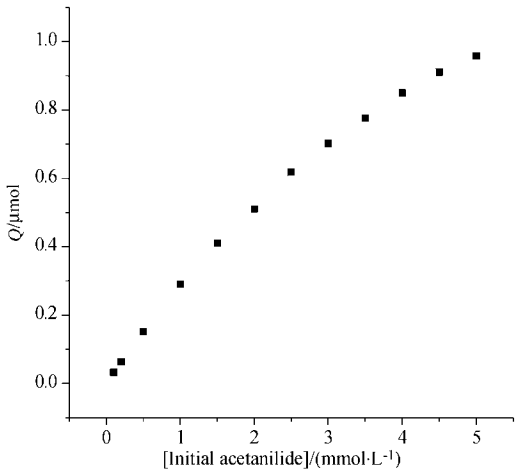

Figure 4 Binding isotherm of P(I). *Q*: amount of acetanilide bound to 20.0 mg of P(I); V=2.0 mL; adsorption time: 12 h.

In this range, the obtained binding data were further processed with Scatchard equation to evaluate the binding parameters of P(I): Q/[acetanilide]=(Q_{max}−Q)/K_D, where Q is the amount of acetanilide bound to MIPs at equilibrium, Q_{max} an apparent maximum number of binding sites and K_D an equilibrium dissociation constant. As shown in Figure 5, the Scatchard plot was straight

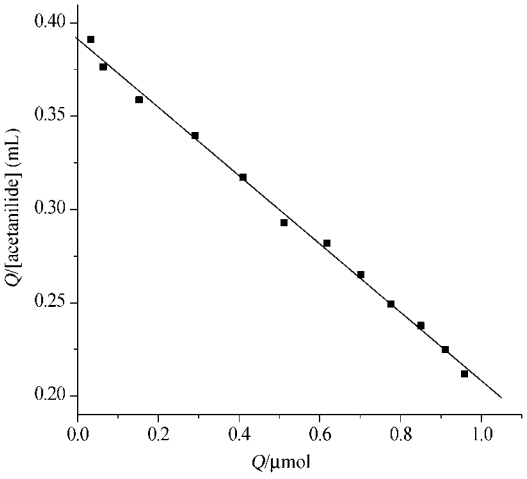

Figure 5 Scatchard plots to estimate the binding nature of P(I). *Q*: amount of acetanilide bound to 20.0 mg of P(I); [acetanilide]: the concentration of free acetanilide.

linear indicating that the binding sites in P(I) were homogeneous with respect to the affinity for acetanilide in the concentration range. K_D and Q_{max} can be calculated to be 5.5 mmol/L and 107.0 μmol/g of dry polymer from the slope and intercept of the Scatchard plot.

Conclusions

In this work, the acetanilide-imprinted polymer was prepared using calix[4]arene derivative **1** and AA as functional monomers. Compared to P(II) prepared using the traditional monomer AA, the new type molecularly imprinted polymers P(I) showed higher sorption capacity to template acetanilide. Attempt to employ calixarenes in molecular imprinting technique may introduce new applications to MIPs and facilitate the probable drug separation and purification.

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(E0307072 ZHAO, X. J.)