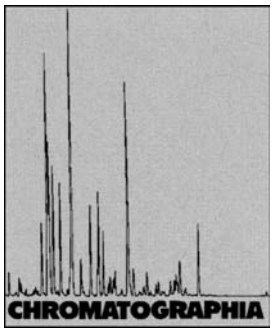
Chromatographic Features and Molecular Recognition Mechanism of a Strychnine Monolithic Molecularly Imprinted Polymer



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Abstract

A monolithic molecularly imprinted polymer (mMIP) with specific recognition ability for strychnine was prepared by in-situ polymerization, using methacrylic acid (MAA) as a functional monomer, ethylene glycol dimethacrylate (EDMA) as a cross-linking agent, toluene and dodecanol as porogenic solvents and 2, 2′-azobisisobutyronitrile (AIBN) as a initiator. Scanning electron microscopy and mercury intrusion porosimetry were used to identify the structural features of the mMIP. The results show that there were three kinds of pore structures. The large through-pore structure allows mobile phase to flow through a column of mMIP with a low back pressure and the other pores lead to the molecular recognition. Some chromatographic conditions such as the pH and the composition of the mobile phase were characterized. Strychnine was separated from compounds such as indole, quinine and brucine. The possible recognition mechanisms were ionic and hydrogen bonding interactions between the strychnine molecule and the mMIP.

Keywords

Column liquid chromatography
Molecularly imprinted polymer
Strychnine
Monolithic column
In-situ polymerization

Introduction

The technique of molecular imprinting was introduced in 1972 by Wulff and Sarhan [1] and much advanced by the work of the Mosbach group in the 1980s [2]. In the past decade, comprehensive reviews and books [3–6] have documented the methods of molecular imprinting which has been shown to be capable of producing materials with 'antibody-like' selectivity. Molecularly imprinted polymers (MIPs) are increasingly used as selective supports in liquid chromatography, capillary electrophoresis, solid-phase extraction [7–11], as biosensors and as artificial antibodies [12, 13]. Non-covalent MIPs can be prepared in several ways, including bulk polymerization [10], in-situ polymerization [15, 16], suspension polymerization [17], multistep-swelling polymerization [18] and grafting polymerization [19, 20]. In order to compare the different polymerization methods, Mayes prepared three types of MIPs by bulk, multistep-swelling and grafting methods when some β-blockers were used as the template molecules. In that study, ground monolithic imprinted polymer was thought to be the best all-round performer for enantiomeric separations of drugs by HPLC [21]. In-situ polymerization has similar recognition ability and possesses the advantages of a one-step preparation procedure and high yield. Matsui and Huang [15, 16] prepared a set of monolithic molecularly imprinted polymers (mMIPs) with cinchonine and amino acid derivatives as the template molecules. Separation of the corresponding enantiomers was achieved but the separation mechanism was not mentioned.

We describe the preparation of an mMIP with a specific recognition for strychnine, The separation mechanism is also discussed.

Experimental

Materials

Methacrylic acid (MAA) was purchased from Tianjin Chemical Reagent Plant (Tianjin, China). Ethylene glycol dimethacrylate (EDMA) was obtained from Aldrich (Milwaukee, USA). Strychnine was purchased from Shanghai No.2 Chemical Reagent Factory (Shanghai, China). Brucine was purchased from Fluka (Switzerland). 2, 2′-azobisisobutyronitrile (AIBN) was

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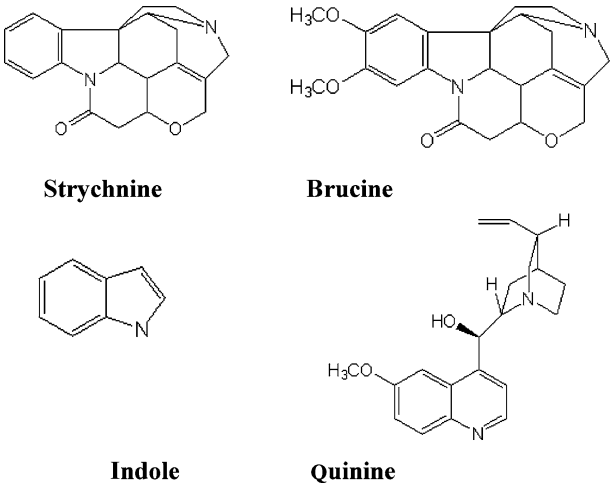

Fig. 1. The structures of compounds used in this study

purchased from Shanghai No.4 Reagent Factory (Shanghai, China). Acetonitrile was of HPLC grade. All the other reagents were analytical grade. MAA was distilled under vacuum to remove any inhibitor before polymerization. EDMA was purified by extraction with 10% aqueous sodium hydroxide and water, and dried over anhydrous magnesium sulfate before use.

Polymer Preparation

The structures of the compounds used in the study are illustrated in Fig. 1. Polymers were prepared by using MAA as the functional monomer and EDMA as the cross-linking agent. The preparation procedure was as follows. Template (0.3 mmol), MAA (1.2 mmol). EDMA (0.47 mmol), toluene (0.37 mL) and dodecanol (1.48 mL) were mixed and degassed by ultrasonication for 10 min. AIBN (0.06 mmol) was added and the mixture was transferred to a 150 mm × 4.6 mm stainless tube sealed at the bottom. Then the top of the stainless tube was sealed in the same manner. The tube was placed in an oven at 45°C for 12 h. A non-imprinted column was prepared in a similar manner without addition of the template molecule. After the polymerization had finished, the plastic seals were removed and the column was connected to the HPLC system. The column was washed with methanol-acetic acid (4:1, *v*/*v*) until a stable baseline at 254 nm was achieved.

Characterization of the Monolithic Molecularly Imprinted Polymer

After the chromatographic experiments had been completed, the column was washed with methanol-acetic acid (4:1, *v*/*v*) for 4 h. The column fitting was removed and the monolithic polymer was pushed out of the column using a flow-rate of 5 mL·min^{-1}. The polymer was dried under vacuum at 40°C for 24 h. The polymer pore size and distribution were determined by mercury intrusion porosimetry (9310 Mercury Porosimeter, USA). Surface analysis of polymer was carried out by using a scanning electron microscope (HITACHI, S-570, Japan) at 20 keV.

High Performance Liquid Chromatography

A Thermo Spectra Series system consisting of a P200 pump and Spectra 100UV-vis detector was used for chromatographic experiments. The data were acquired and processed with Anastar chromatographic software (Tainjing, Dongkang); An LKB 2219 multitemp thermostatic circulator (Bromma, Sweden) was used to control the column temperature at 25°C. Eluent flow-rate was 0.5 mL·min^{-1} or 1.0 mL·min^{-1} with detection at 254 nm. Standards (1 mg·mL^{-1} in ethanol, 10 μL) were injected onto the column by using filled-loop injection. The void volumes were determined by injection of acetone. The retention factors (k) were calculated according to the standard chromatographic theory: $k = (t_R - t_0)/t_0$, where t_R is the retention time of the solute, t_0 is the retention time of the void marker.

Results and Discussion

Evaluation of the Imprinted Polymer

Figure. 2a shows the SEM image and 2b the pore size distribution profile of the strychnine mMIP under optimized conditions. The results reveal that large through-pores are present and the mobile phase is able to flow through with low resistance at high flow rates. The pore size distribution profile shows that the average pore size is 500 nm in the strychnine mMIP. Figure. 3a shows that the four alkaloids can be separated on the strychnine mMIP when the mobile phase is a mixture of acetonitrile-acetic acid. Figure. 3b shows that the baseline separation of brucine and strychnine is achieved when the mobile phase is 25 mmol·L^{-1} phosphate buffer-acetonitrile and stepwise gradient elution is used. These experiments indicate that the strychnine mMIP has specific recognition for the strychnine molecule.

Influence of the Mobile Phase Composition on Retention of Strychnine

Influence of Acetic Acid in Mobile Phase

Figure. 4 shows the influence of acetic acid on retention of strychnine and brucine using acetonitrile as mobile phase. The retention factors of strychnine and brucine decrease when the amount of acetic acid increases from 4% to 8%. These effects are believed to be caused mainly by the desovlation required on the interactions between the strongly solvated ammonium and carboxyl groups [22]. The addition of acetic acid leads to the protonation of the functional carboxyl groups of the mMIP. Thus the ionic interactions between the stationary phase and the solute are weakened, resulting in a decreased k. On the other hand, the addition of acetic acid destroys the hydrogen bonds between the carbonyl groups and the ether bonds of the molecule.

Influence of the Mobile Phase pH

Figure 5 shows that the retentions of strychnine and brucine increase with an

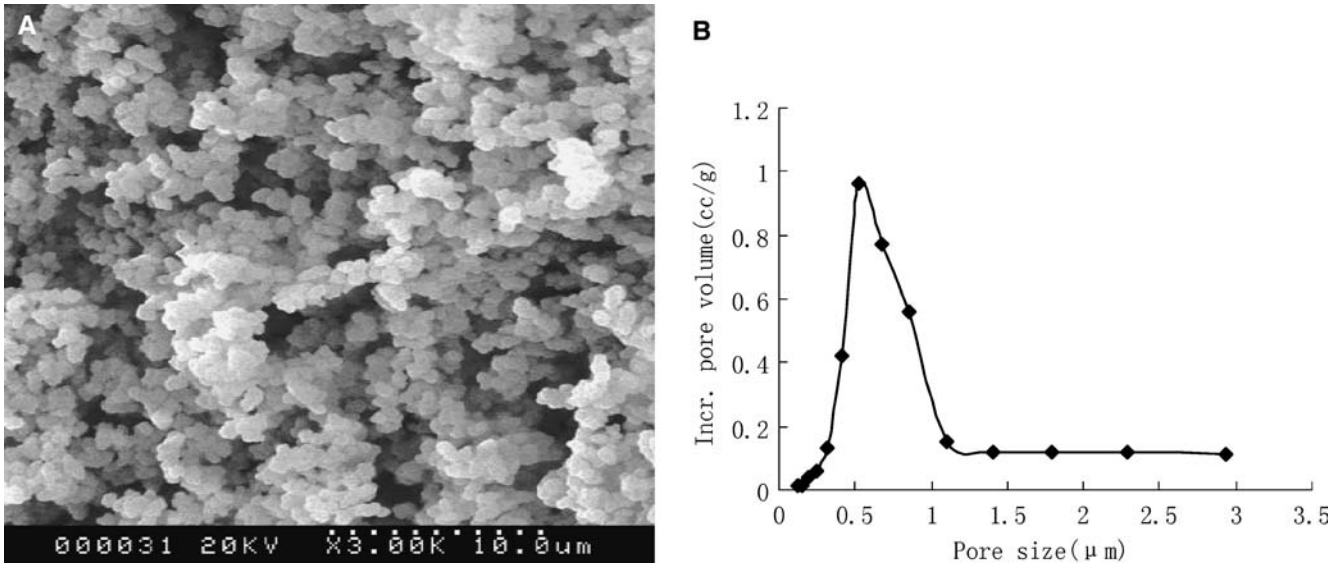

Fig. 2. SEM and Pore size distribution profile images of the strychnine mMIP (**A**). scanning electron micrograph, (**B**) pore size distribution profile

increase in pH when phosphate buffer (25 mmol·L^{-1}) and acetonitrile (50:50, *v*/*v*) are used as mobile phase. MAA has been used for the preparation of MIPs for many template molecules [23–26] as a functional monomer, largely due to the potential for the hydrogen bonding and ionic interactions that occur in carboxyl groups. As seen in Fig. 5, the maximum in retention of strychnine on the monolithic non-imprinted polymer (mNIP) occurres at pH 7.0. However, we could only get retention factors of strychnine on the mMIP within the range of pH 3 to 5 because its retention on the mMIP was too strong to be eluted with the aqueous buffer (pH > 5). Shea and Sellergren believe that pKa value of the mNIP is higher than that of the mMIP and that the maximum in retention occur at pH values corresponding to the apparent pKa values of the solutes [27]. There are two nitrogen atoms in the molecules of strychnine and brucine, and the pKa of each molecule is 8.0. So the maximum in retention of strychnine on the mMIP should occur in the aqueous buffer at around pH 7.0. These results indicated that ionic interactions play an important role in the chromatographic process and also fit the model described by Shea and Sellergren [27].

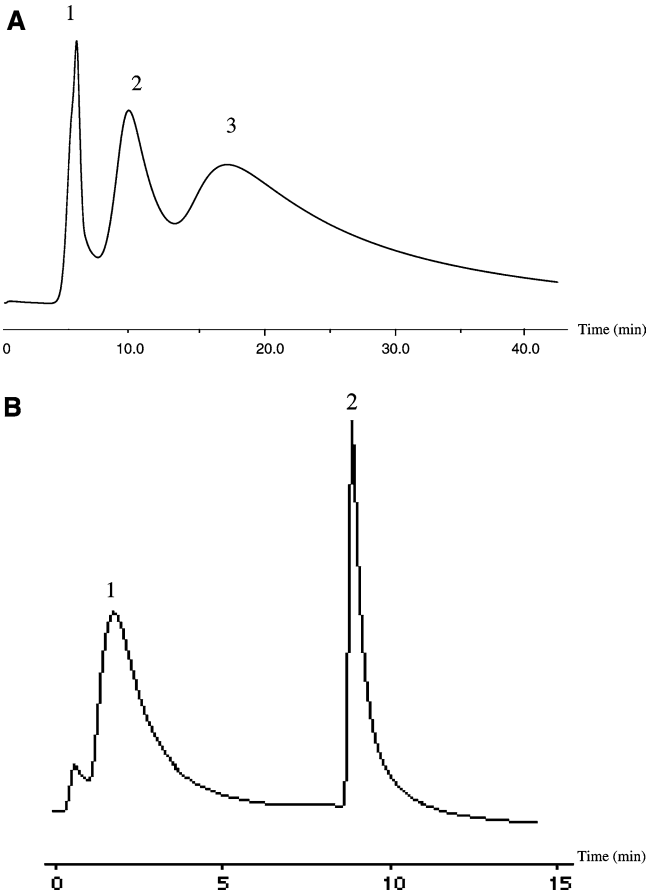

Fig. 3. Chromatogram of several alkaloids on the strychnine mMIP
(**A**) Isocratic elution at 0.5 mL·min^{-1} with acetonitrile-acetic acid (96:4 *v*/*v*) mobile phase; **1** = indole, quinine; **2** = brucine; **3** = strychnine (**B**) stepwise gradient elution at 1.0 mL·min^{-1}, 0–7 min, 25 mmol phosphate buffer (pH 5.0)-acetonitrile(50:50, *v*/*v*), 7–15 min 25 mmol phosphate buffer (pH 2.0) –acetonitrile (50:50, *v*/*v*), **1** = brucine **2** = strychnine

Influence of the Proportion of Buffer in the Mobile Phase

Figure 6 shows the effect of the amount of the buffer in the mobile phase on the retention of strychnine and brucine. With an increasing amount of phosphate buffer in the mobile phase and a pH value of the phosphate buffer below 4.6, (the pK_a of MAA), the retentions of strychnine and brucine decreases. This is because the carboxyl groups of the mMIP cannot dissociate and the hydrophobic interaction with the backbone of the polymer are decreased. The structures of strychnine

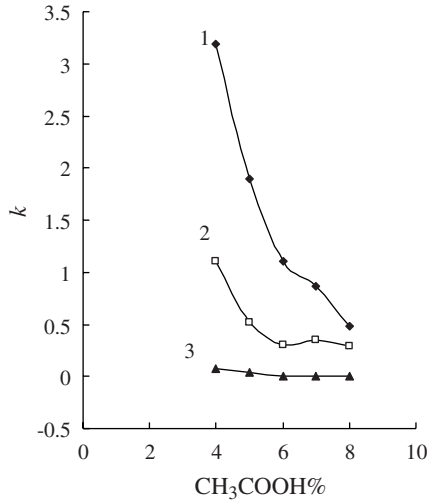

Fig. 4. Influence of the amount of acetic acid in the mobile phase on the retention of strychnine and brucine. HPLC conditions: MIP column size: 150 mm × 4.6 mm, Mobile phase: acetonitrile-acetic acid (*v*/*v*), flow-rate: 0.5 mL·min^{-1}, detection: 254 nm, ♦ strychnine; □ brucine; ▲ strychnine (on the non-imprinted column)

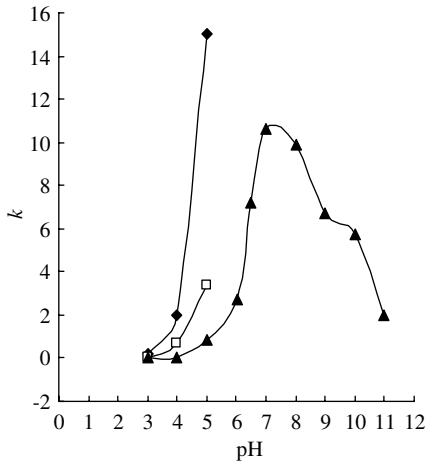

Fig. 5. Influence of the pH of the mobile phase on the retention of strychnine and brucine HPLC conditions: MIP column size: 150 mm × 4.6 mm, Mobile phase: acetonitrile-25 mmol phosphate buffer (50:50, *v*/*v*), flow rate: 1.0 mL·min^{-1}, detection: 254 nm. ♦ strychnine; □ brucine; ▲ strychnine (on the non-imprinted column)

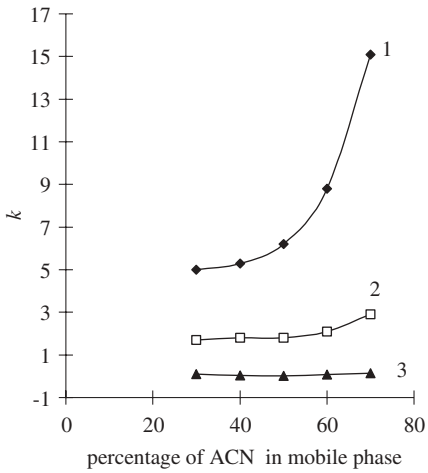

Fig. 6. Effect of the proportion of buffer in the mobile phase on the retention of strychnine and brucine. HPLC conditions: MIP column size: 150 mm × 4.6 mm, Mobile phase: acetonitrile-25 mmol phosphate buffer (*v*/*v*), flow rate: 1.0 mL·min^{-1}, detection: 254 nm. ♦ Strychnine; □ Brucine; ▲ strychnine (on the non-imprinted column)

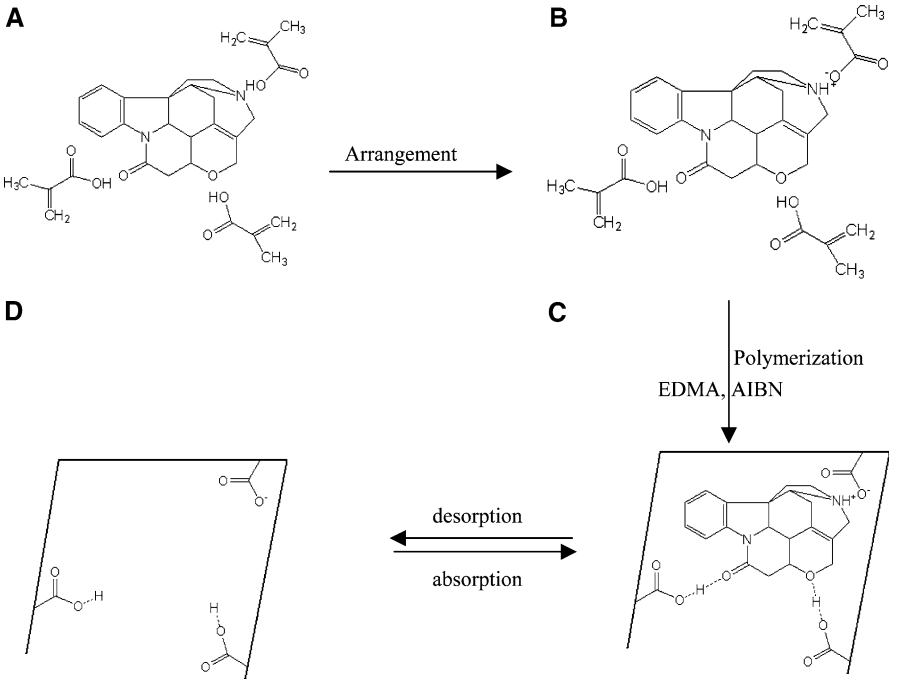

Fig. 7. Schematic model of strychnine molecule imprinting mechanism. (**A**) strychnine and monomer(MAA) were allowed to form solution complexes (**B**) the complex was fixed using a cross-linking agent (EDMA) (**C**) locking the complexes in position in the resulting material. (**D**) recognition sites which are selective for the template structure

and brucine are similar as far as hydrophobic interaction is concerned, but when the template of the polymer is strychnine, the cavities in the polymer match the strychnine molecule better than the brucine molecule. As a result, the proportion of the buffer in the mobile phase has less effect on the retention of brucine.

Interactions between Template Molecule and Functional Monomer

Based on our results, we believe that the template molecules interact with the carboxyl groups of monomer by ionic interaction. Furthermore, hydrogen bonds arise between the carbonyl groups and the ether bonds of the molecules [22]. The schematic model of the strychnine imprinting mechanism is shown in Fig. 7. This is in agreement with previous studies carried out by Nittsson [28] which have already proved that ionic interactions arised between the fatty amine and carboxylic acid, and hydrogen bonds generally exist between the carboxyls of the organic compound.

Conclusion

A strychnine mMIP was successfully prepared by using an in-situ technique, using MAA as the functional monomer and EDMA as the cross-linking agent. The strychnine mMIP shows specific recognition ability for the strychnine molecule. One of the major interactions between the strychnine molecule and mMIP is ionic interaction. The same binding sites on the mMIP exhibit a different bonding strength for the solute molecules when the pH and the composition of the mobile phase are changed.

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