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Preparation of molecularly imprinted polymer for sinomenine and study on its molecular recognition mechanism

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Abstract

A sinomenine (SIN) molecule-imprinted monolithic stationary phase (MIMSP) with specific recognition for SIN was prepared by in situ technique, utilizing methacrylic acid (MAA) as a function monomer, ethylene glycol dimethacrylate (EDMA) as a cross-linking agent, and low-polar solvents (toluene and dodecanol) as porogenic solvents. The selectivity of the polymers for SIN was evaluated by high performance liquid chromatography (HPLC). Some chromatographic conditions, such as the column temperature, the flow rate and the composition of the mobile phase, were changed in order to characterize the chromatographic procedure. SIN could be separated from some other structural analogues, including morphine, codeine, codethyline and magnoflorine, under optimized conditions. Scatchard analysis showed that two classes of binding sites existed in the SIN-imprinted polymers, with their dissociation constants estimated to be 7.257×10^{-5} and 3.828×10^{-3} mol l^{-1}, respectively. Compared with the SIN-imprinted polymers, the non-imprinted polymers prepared using the same method but in the absence of SIN did not exhibit the specific molecular selectivity, which suggests that the specific molecule-recognition ability of the SIN-imprinted polymers was largely ascribed to the imprinting effect.

Keywords: Molecularly imprinted polymer; Sinomenine; In situ technique

1. Introduction

Molecularly imprinted technique was introduced in 1972 by Wulff and Sarhan [1] and much advanced by the work of the Mosbach group in the 1980s [2]. This technique has been shown to be capable of producing materials with 'antibody-like' selectivity. Because molecularly imprinted polymers (MIPs) have predetermined selectivity, recognition and feasibility, they have been used in many fields. They are increasingly being used as selective supports in liquid chromatography, capillary electrophoresis, and solid-phase extraction, and as catalysts, bionic sensors and artificial antibodies [3–8].

MIPs can be prepared by both covalent and non-covalent methods. Non-covalent methods include bulk polymerization [9], in situ polymerization [10–12], suspension polymerization [13], and multistep-swelling polymerization [14]. Compared with other methods, in situ polymerization possesses several advantages, e.g. simple preparation procedure. Matsui and Huang [10–12] prepared MIMSP using cinchonine and amino acid derivatives as the template molecules, by which the rapid separation of their diastereomers and enantiomers was achieved. Recently, a monolithic MIPs with specific recognition ability for strychnine has been synthesized in our lab, and the molecular recognition mechanism was discussed [15].

Sinomenine (SIN) is one of the principal alkaloids isolated from *Sinomenium acutum Rehd. et Wils*. It has analgesic and anti-inflammatory effects, and is used clinically to cure rheumatoid arthritis and neuralgia [16]. So far SIN has been extracted from herbs mainly using aether or toluene as extraction solvent [17–19], with little attention to operation safety or environmental protection. Recently, the general determination methods of SIN have been thin-layer chromatographic scanning [20], HPLC [21], etc. whose selectivity and specificity are not good enough to accommodate the variety and complexity of biological samples. For these reasons, it is necessary to develop an effective method to extract SIN from herbs and biological fluids. In this paper, we prepared a SIN molecule-imprinted stationary phase (SIN-MIMSP) by in situ method, which shows specific recognition ability for the template molecule, i.e. SIN. Furthermore, we explored the

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possible recognition mechanism of the polymer by HPLC and Scatchard analysis.

2. Experimental

2.1. Materials

Methacrylic acid (MAA) was purchased from Tianjin Chemical Reagent Plant (Tianjin, China). 2-(Trifluoromethyl)-acrylic acid (TFMAA) and ethylene glycol dimethacrylate (EDMA) were obtained from Aldrich (Milwaukee, USA). 2,2′-Azobisisobutyronitrile (AIBN) was purchased from Shanghai No. 4 Reagent Factory (Shanghai, China). SIN was purchased from Shaanxi Scidoor HI-tech Biology Co. Ltd with a labeled purity above 99.0% (Shaanxi, China). Morphine, codeine, codethyline and magnoflorinea were obtained from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). The structures of these compounds are illustrated in Fig. 1. Acetonitrile was of HPLC grade. All the other reagents were of analytical grade. MAA was distilled under vacuum to remove the inhibitor before polymerization. EDMA was purified according to the report [22]. Water was freshly distilled three times prior to use.

2.2. Preparation of polymers

Polymers were prepared by utilizing MAA as the functional monomer and EDMA as the cross-linking agent. The preparation procedure was as follows. Template (0.2 mmol), toluene, MAA, EDMA, dodecanol and AIBN were mixed and degassed by ultrasonication for 10 min. The mixture was purged with nitrogen for 5 min and then transferred into a stainless-steel column (100 mm×4.6 mm i.d.). The column was sealed and the mixture was kept at a certain temperature for 12 h. The resulting polymers were washed by using a mixture of methanol-acetic acid (4:1, v/v) to remove the template molecule, and then the residual acetic acid was removed using methanol. Non-imprinted polymers were prepared by the same procedure without the addition of template molecule.

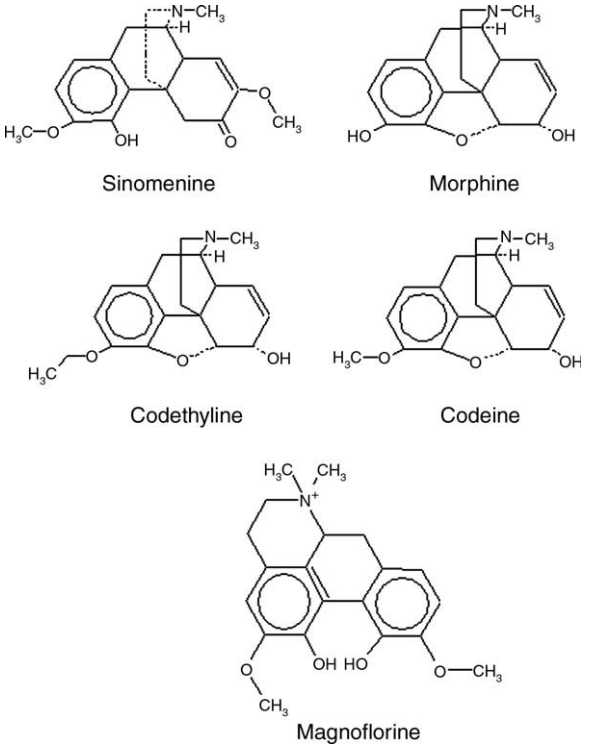

Fig. 1. Structures of sinomenine and its structural analogues.

2.3. Test of the morphologies of polymers

The morphologies of the MIP were analyzed by using a scanning electron microscope (HITACHI, S-570, Japan) at 20 keV, and the pore properties were determined by mercury intrusion porosimetry (9310 Mercury Porosimeter, USA).

2.4. Chromatography

The HPLC system was composed of a Spectra P200 pump, a Spectra 100 UV detector (Thermo Electron Co., Boston, USA) and an Anastar Chromatographic software. Detection was performed at 262 nm. The eluent used was specified in the legends of tables and figures. The retention factors were determined by the relation $k=(t_R-t_0)/t_0$, where t_R is the retention time of a given species and t_0 is the retention time of the void marker (acetone). The selectivity factors were calculated from the equation $S=k_{imprinted}/k_{non\text{-}imprinted}$, where $k_{imprinted}$ and $k_{non\text{-}imprinted}$ were the retention factors of SIN on the molecularly imprinted and non-imprinted polymers, respectively. The separation factors were calculated from the equation $\alpha=k_1/k_2$, where k_1 and k_2 were the retention factors of SIN and its analogues on the SIN-MIMSP, respectively.

2.5. Scatchard analysis

The polymers were pushed out of column. After that, 20 mg of the polymers were weighed into a 10 ml conical flask and mixed with 5.0 ml of SIN aqueous solution, the concentration of which varied from 0.1 to 4.5 mmol l^{-1}. The flasks were oscillated by an HZ-881S action shaker (Taicang City Scientific Instruments Factory, China) in a water bath for 16 h at 25 °C. Then the mixture was filtrated through a microporous membrane of 0.22 μm and the SIN concentration in the filtrate was measured by a SP-2102 UV (Shanghai Spectrum Instruments Co., Ltd, China) at 262 nm. The amount of SIN bound to the polymers was calculated by subtracting the concentration of free SIN from the initial SIN loading. The Scatchard equation $Q/[SIN]=(Q_{max}-Q)/K_d$ was used to estimate the binding parameters of the SIN-imprinted polymers, where Q was the amount of SIN bound to the polymer, Q_{max} was the apparent maximum number of binding sites, K_d was the equilibrium dissociation constant, and [SIN] represented the equilibrium concentration of SIN.