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Mechanism of molecular recognition on molecular imprinted monolith by capillary electrochromatography

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Abstract

The recognition mechanism of molecularly imprinted polymer (MIP) in capillary electrochromatography (CEC) is complicated since it possesses a hybrid process, which comprises the features of chromatographic retention, electrophoretic migration and molecular imprinting. For an understanding of the molecular recognition of MIP in CEC, a monolithic MIP in a capillary with 1,1′-binaphthyl-2,2′-diamine (BNA) imprinting was prepared by in situ copolymerization of imprinted molecule, methacrylic acid and ethylene glycol dimethacrylate in porogenic solvent, a mixture of toluene-isooctane. Strong recognition ability and high column performance (theory plates was 43,000 plates/m) of BNA were achieved on this monolithic MIP in CEC mode. In addition, BNA and its structural analogue, 1,1′-bi-2, 2′-naphthol, differing in functional groups, were used as model compounds to study imprinting effect on the resultant BNA-imprinted monolithic column, a reference column without imprinting of BNA and a open capillary. The effects of organic modifier concentration, pH value of buffer, salt concentration of buffer and column temperature on the retention and recognition of two compounds were investigated. The results showed that the molecular recognition on MIP monolith in CEC mode mainly derived from imprinting cavities on BNA-imprinted polymer other than chromatographic retention and electrophoretic migration.

Keywords: Molecularly imprinted polymer; Monolith; Capillary electrochromatography; 1,1′-Binaphthyl-2,2′-diamine; Recognition mechanism

1. Introduction

The development of systems capable of mimicking the molecular level selectivities observed in nature has been the focal point for intense research interest over recent decades. Numerous model systems have evolved that mimic the interaction between a substrate (the guest) and a receptor (the host). One attractive approach to study/mimic nature is molecular imprinting technology (MIT) [1–5]. By this approach, the resultant synthetic polymer, i.e., molecular imprinting polymers (MIPs), demonstrates remarkable selectivities for the used template molecule. An assembly of functional monomers around a template molecule is organized by either covalent bonds and/or non-covalent forces such as hydro-bonding, electrostatic, and hydrophobic interactions. Polymerization of a solution containing the assembly, cross-linker and inert solvent results in a highly cross-linked network polymer. After the removal of the template from the polymer, left in the polymer matrix are three-dimensional cavities that possess a "memory" for the used template molecule in terms of complementarity of both shape and chemical functionality. MIPs can recognize the template molecule by the binding sites in the cavities. The advantages that molecularly imprinted polymers (MIPs) possess over biopolymers are low cost, good physical and chemical stability. Recently, MIPs have found application in an ever increasing range of application areas, such as enzyme-like catalysis [1], bio-mimetic sensors [2], antibody mimics [3], solid-phase extraction [4], and chromatography [5].

The resultant MIPs are usually evaluated by high performance liquid chromatography (HPLC). However, low column performance of MIPs stationary in HPLC mode limits the application of MIPs. Capillary electrochromatography

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(CEC) has during the last decade been exposed to much research since this technique shows great promise for analytical separation. CEC is considered to combine the advantage of the high separation efficiency of capillary electrophoresis and high selectivity offered by HPLC. CEC-based MIPs have shown higher efficiency than HPLC-based MIPs. In addition, another attractive is miniaturized format of CEC, thus fewer templates or monomers for the molecular imprinting will be consumed, which is especially valuable to those expensive chemicals. Up to date, the utility of the MIP-based technology in CEC is still limited and there are three different MIPs formats for CEC: (1) the particle [6–10], (2) the coating [11–14] and (3) the monolith [15–21], which seems to be a new trend in chromatography.

The imprinting process is commonly believed to result in the formation of shape-complementary microcavities with defined spacial arrangement of functional groups. However, the interaction mechanisms of specific rebinding of the template have been studied less. The interaction mechanism has proved to be ion-exchange [22] or hydrogen bonding mechanism [23]. It was believed that imprinted polymers in different environments could show recognition properties based on different molecular interaction [24]. Especially, to an MIP that has been synthesized in the presence of an organic solvent and later evaluated in an aqueous environment, the contribution of the different forces involved in binding can change dramatically. In CEC-based MIP, the retention process were interplay of multiple mechanisms of ion-exchange, electrophoresis and molecular imprinting [25], in which the molecular recognition is more profound.

For investigating the mechanism of molecular recognition of MIPs in an aqueous environment, a mode analyte, 1,1-binaphthyl-2,2′-diamine (BNA), was selected as a template and a monolithic MIPs in a capillary was synthesized. There is no report for the preparation of an MIP for BNA. The retention and recognition of BNA and its analog, 1,1′-binaphthol (BINOL) (see Fig. 1), were studied in the MIP monolith in a CEC mode.

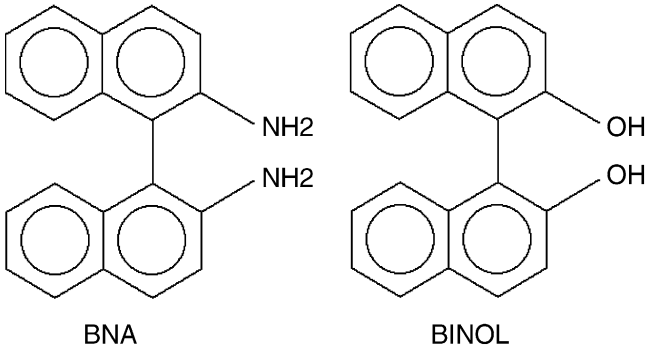

Fig. 1. The structure of BNA and BINOL.

2. Experimental

2.1. Reagents and chemicals

3-(Trimethoxysilyl) propyl methacrylate (γ-MPS) was from Acros (Geel, Belgium). Methacrylic acid (MAA) was from Beijing Donghuan Chemical Reagent (Beijing, China). Ethylene glycol dimethacrylate (EDMA) was from Suzhou Anli Chemical & Engineering Co. (Suzhou, China). 2,2′-Azobis (2-isobutyronitrile) (AIBN) was supplied by Special Chemical Reagent Factory of Nankai University (Tianjin, China). BNA was from Acros (Geel, Belgium). BINOL was supplied by Nankai Biology Co. (Tianjin, China). HPLC-grade acetonitrile (ACN) was supplied by the Tianjin Chemical Reagent (Tianjin, China). Other analytical reagents were from Tianjin Chemical Reagent Co. (Tianjin, China). Fused-silica capillaries with 100 μm I. D. and 375 μm O.D. were purchased from Yongnian Optic Fiber Plant (Hebei, China).

2.2. Preparation of MIP capillary columns

A fused-silica capillary was flushed with 1 M NaOH followed by water for at least 30 min each. Then the capillary was filled with a solution of 4 μL of γ-MPS in 1 mL of 6 mM acetic acid, and the solution was kept in the capillary for 1.5 h. The capillary was then flushed with water and dried with a flow of nitrogen. A pre-polymerization mixture containing imprinted molecules, functional monomer (MAA), cross-linking monomer (EDMA) and radical intiator (AIBN) dissolved in toluene or toluene-isooctane, composed as described in Table 1. Optimized pre-polymerization mixture observed was composed of MAA (41 μL), EDMA(362 μL), toluene (622 μL), isooctane (156 μL), BNA (17.54 mg) and AIBN (3.6 mg). The pre-polymerization mixture was sonicated for 10 min and introduced to the capillary. The ends of the capillary were sealed with soft plastic rubber. The cap-

Table 1
Preparation protocol for MIP monolith

Column label	Imprinted species	*C* (M)	MAA (M)	EDMA (M)	Isooctane (v/v, %)	I/M (mol/mol)	M/E (mol/mol)
A	BNA	0.0605	0.484	1.936	0	1:8	1:4
B	BNA	0.121	0.484	1.936	20	1:4	1:4
C	BNA	0.0605	0.484	0.968	20	1:8	1:2
D	BNA	0.0605	0.484	1.936	20	1:8	1:4
E	BNA	0.0605	0.484	2.420	20	1:8	1:5
F	BNA	0.0605	0.484	1.936	10	1:8	1:4
G	None	–	0.484	1.936	20	–	1:4

I, imprinted molecule; M, MAA; E, EDMA.