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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.

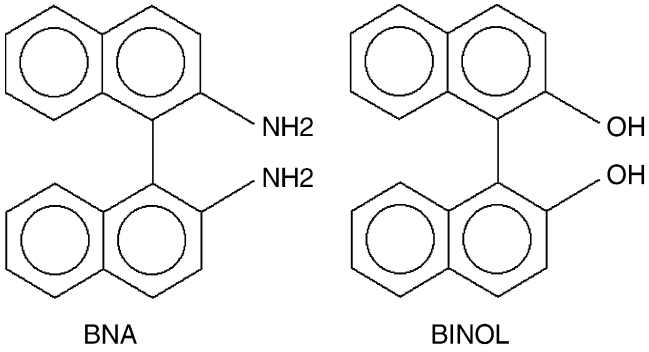

Fig. 1. The structure of BNA and BINOL.

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.

illary was submerged in a 60 °C water bath for 3 h. After polymerization, the capillary was flushed with acetonitrile and electrolyte, respectively, using a hand-held syringe to remove any unreacted reagents. A detection window was created at the end of the continuous polymer bed (at a distance of about 1 mm) by burning out 2–3 mm segment of the polyimide outer coating. A blank capillary column without imprinted molecule was prepared in the same way (see Table 1).

2.3. Capillary electrochromatography

Electrochromatographic experiments were carried out on a Beckman P/A CE MDQ system (Beckman, Fullerton, CA, USA) equipped with a P/A CE system MDQ UV detector. An IBM personal computer with Beckman P/A CE system MDQ capillary electrophoresis software was used. The total length of the capillary was 31.2 cm and effective length (MIP-based stationary phase) was 20 cm. The column temperature was kept at 25 °C. A pressure of 20 psi was applied to both vials during the separation. The electrolyte was a mixture of acetonitrile and different ratios of buffer with different pH. All the buffer was made using double distilled water and filtered with 0.2 μm membrane. Separation was performed at 15 kV.

In this paper, separation factor is evaluated using α', which is calculated by

$$\alpha' = \frac{t_2}{t_1}$$

because some of analytes are eluted prior EOF, t_1 and t_2 are the retention times of the first and second peaks. The resolution (R_s) was calculated by

$$R_s = \frac{t_2 - t_1}{0.5(W_2 + W_1)}$$

W is the width at the baseline between tangents drawn to inflection points for the peak.

3. Results and discussion

3.1. Preparation of MIP monolith and column repeatability

The key to successful column preparation of MIP monolith is choice of the composition of the pre-polymerization mixture, i.e., nature and quality of porogen, the content of cross-linking monomer and the ratio of imprinted molecule to functional monomer.

Porogenic solvent plays a dual role in preparation of MIP monolithic column. First, the porogen should produce large pores to assure good flow-through properties of the resultant MIP. Second, The porogenic solvent governs the strength of non-covalent interactions in addition to its influence on the polymer morphology. Toluene has proved as a good porogen for molecular imprinting [15]. However, in our study, good column permeability cannot be obtained with toluene as porogen. Isooctane is regarded as a porogen for imprinting not interfering with imprinting process and also produced good column permeability. In our study, 20% (v/v) isooctane in porogens was found optimum for both solution of imprinted molecule and non-covalent interactions of imprinted molecule and functional monomer.

In contrast to the effects of porogenic solvent, variations in the monovinyl/divinyl monomer ratio not only produce different porous structures but also leads to imprinted polymers with different compositions. A higher content of divinyl monomer (EDMA) results in more highly cross-linked MIPs. Higher cross-linking favors the rigidity to preserve the structure of the cavity after splitting off the template. Unfortunately, in our work, when the content of cross-linking monomer is its high level (e.g., the ratio of MAA-to-EDMA is 1:5), the resultant MIP monolith was very dense. As a result, it was not possible to the exchange of the solvent of polymerization for an electrolyte, and to evaluate column further in CEC mode. We also investigated low level of EDMA but little molecular recognition observed on the resultant MIP column in spite of good column permeability. Optimum ratio of MAA-to-EDMA found was 1:4.

The ratio of imprinted molecule to functional monomer also affects the preparation of MIP monolith. Imprinted polymers formed using a noncovalent strategy rely on the solution concentration of functional monomer to form the pre-polymer complex (PPC). By Le Chatlier's principle, increasing concentration of imprinted molecule should increase the concentration of PPC. However, a higher ratio, i.e., BNA/MAA = 1:4, rendered a higher local concentration of the imprinted molecule and accelerated the speed of polymerization. Thus, it is difficult to pump liquid through the capillary column. Optimum ratio of imprinted molecule-to-functional monomer found was 1:8.

The EOF velocities as measured with 80% acetonitrile in the mobile phase varies only 1.9% within 30 days and after 200 electrochromatographic runs. RSD values of retention factor of imprinted molecule is below 5%. Beyond 100 injections, molecular recognition derives from molecular imprinted can still realized. The MIP monolithic format does exhibit exceptional stability that is attributed to the absence of moveable particle in the column packing and the lack of need for retaining frits in the MIP monolithic column. The strong bonding between the silanized innerwall and the porous MIP enhances the stability of the MIP column also.

3.2. EOF Characterization of MIP monolith

In noncovalent imprinting, MAA is commonly used as a functional monomer, which can act as a hydrogen donor or acceptor. In the MIP monolith, MAA is the only ionizable monomer and thus provides the fixed charged sites for the generation of EOF that CEC needs. Due to the dual role of

MAA in MIP monolithic matrix, conditions that affect the EOF also affect the retention and the recognition of imprinted molecule. Therefore, knowing the characteristics of EOF will be helpful to the understanding of separation behavior and the mechanism in CEC.

The electroosmotic mobility can be expressed by:

$$\mu_{eo} = \frac{v_{eo}}{E} = \frac{\delta\sigma}{\eta}$$

where μ_{eo}, v_{eo}, E, δ, σ, η, are the electroosmotic mobility, the EOF velocity, the external electric field strength, the electrical double-layer thickness, the amount of charge per unit surface area in the stern plane the number of valence electrons and the electrolyte viscosity, respectively. The effect of factors on the electroosmotic mobility were studied, including the content of acetonitrile, pH, concentration of salt in the mobile phase using thiourea as an EOF marker.

The effect of the content of acetonitrile on the EOF is investigated. The effects of acetonitrile content on the EOF in the range of 80–95% (v/v) and 0.05 M acetate (pH 5.0) is shown in Fig. 2A. The content of acetonitrile in the mobile phase influences the EOF mobility through the ratio of permittivity to viscosity, ε_r/η. In the range of 80–95%, ε_r/η increases with the increase of the content of acetonitrile [26], thus the EOF mobility increases.

The effect of pH on the EOF is shown in Fig. 2B. The EOF on BNA-imprinted monolith is mainly generated by the dissociated functionalities of the MAA. This leads to pH-dependent EOF, due to the relatively high p*K*a, i.e., between 6 and 7. With the increase of pH at the pH range of 3.0–6.0, the ionization content of MAA is increased and as a result, the EOF mobility increased. It is observed from Fig. 2B that when value of pH was 6.0, the EOF of the MIP monolith reaches $1.00 \times 10^{-8}\ m^2\ V^{-1}\ s^{-1}$, which is much higher than

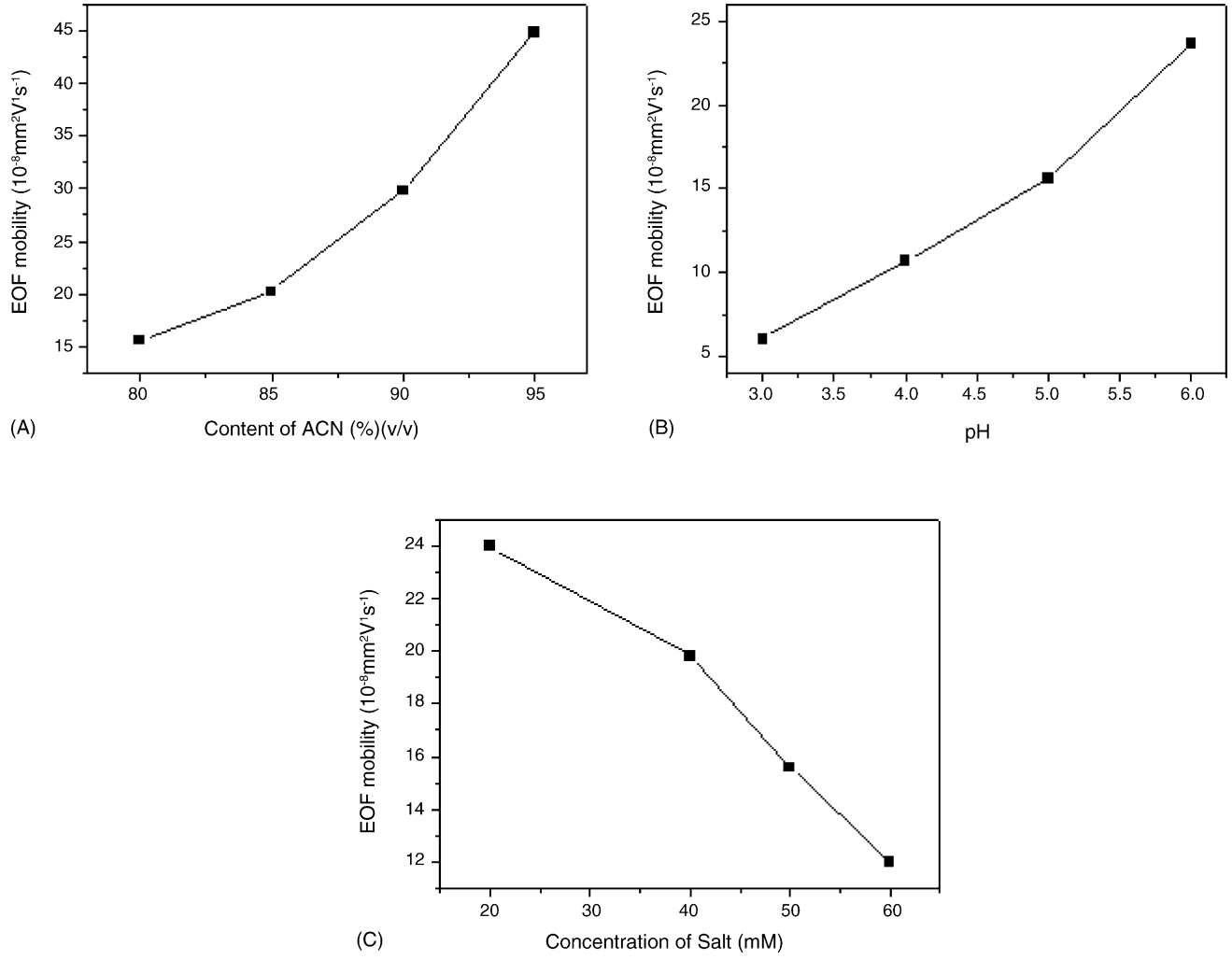

Fig. 2. Effect of CEC parameter on the EOF mobility measured with thiourea as the unretained neutral marker on the MIP monolithic column. The capillary was thermostated to 25 °C and UV detection was carried out at 254 nm. Separation was performed on the MIP monolith at 15 kV and over-pressure of 20 psi. (A) EOF mobility against the acetonitrile concentration in the eluent. The electrolyte used was composed of acetonitrile (80–95%, v/v)/0.05 M acetate (pH 5.0) (20–5%, v/v). (B) EOF mobility against the pH value in the eluent. The electrolyte used was composed of acetonitrile/0.05 M acetate (pH 3.0–6.0) (80/20, v/v). (C) EOF mobility against the acetate concentration in the eluent. The electrolyte used was composed of acetonitrile/acetate (pH 5.0) (80/20, v/v) (0.02–0.06 M).

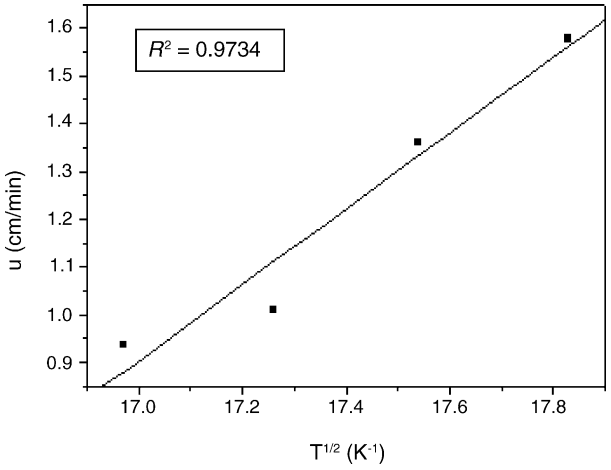

Fig. 3. Effect of temperature on the EOF mobility measured with thiourea as the unretained neutral marker on the MIP monolith. The column temperature was increased from 15 to 45 °C in 10 °C increments. CEC was performed at 15 kV and over-pressure of 20 psi. The electrolyte used was composed of acetonitrile/acetate (pH 4.0, 50 mM) (80/20, v/v). UV detection was carried out at 254 nm.

pH 3.0. This is in agreement with polymer-based monolithic column in which MAA was used as a EOF promoter [27].

The effect of salt concentration on the EOF is studied using different ionic strength of electrolyte from 0.02 to 0.06 M acetate (pH 5.0)/acetontrile (20/80, v/v) (see Fig. 2C). The EOF is decreased by increasing the salt concentration at a constant content of acetonitrile (80%). This can be explained by the relatively smaller thickness of the electrical double layer at higher ionic strength.

The effect of temperature on the EOF was studied by varying the temperature of MIP monolithic column from 15 to 45 °C (Fig. 3). Temperature has a profound influence on the EOF. Increased temperature reduces mobile phase viscosity via the exponential relation [28] and hence increases μ_{eo}, so that, for a given voltage, more rapid separation is possible.

3.3. Retention of the imprinted molecule on the MIP monolith

Retention data may be very useful to understand the mechanism of rebinding of imprinted molecules. The effect of the content of acetonitrile in the mobile phase on the retention was investigated by plotting retention time versus content of acetonitrile (Fig. 4A). There was a sharp decrease in the retention of BNA and BINOL on BNA imprinted monolith when the content of acetonitrile was changed from 80 to 95%. At values lower than 80% (v/v) acetonitrile, the viscosity of the mobile phase increased while the eluent strength decreased, thus lowing the mobile phase flow velocity and increasing solute retention (>70 min in retention time).

The effect of pH on the retention was studied using different pH value from pH 3.0 to 6.0 (Fig. 4B). With the increase of pH, the trend of the retention of BNA and BINOL on MIP column decreases due to the increase of EOF. It is worth noting that almost no retention differences between BNA and BINOL at pH 6.0 is observed. As discussed earlier, the carboxylic acid groups of the selective sites in the imprinted polymer have a lower average p*K*a than those of the non-selective sites [22]. With an increase of pH, non-selective sites increased faster than selective sites resulting stronger non-selective interactions. As a result, in additional to the contribution of EOF, these non-selective interaction between analytes and MIPs ought be responsible for the decrease in selectivity observed at high pH value.

The effect of salt concentration on the retention was studied using different concentration of buffer with 20–60 mM acetate (pH 5.0)/acetonitrile (20/80) (Fig. 4C). With the increase of salt concentration, the retention time of BNA and BINOL decreased.

The effect of temperature on the retention was studied by varying the temperature of MIP monolithic column from 15 to 45 °C (Fig. 5A). With the increase in temperature, the retention time of BNA and BINOL was sharply decreased. Apart from the increase in EOF, the retention of BNA and BINOL is also influenced by increasing column temperature because of the increased partition into the mobile phase.

The migration of charged solutes in CEC is the result of both their electrophoretic mobility and chromatographic retention on the stationary phase. Thus, the migration velocity, u_m, of a charged analyte is expressed by the sum of the velocity of the mobile phase, u_{eo}, and the electrophoretic velocity of the analyte, u_{ep}, multiplied by the retardation factor ($1/1 + k^*$), as follow:

$$u_m = \frac{u_{ep} + u_{eo}}{1 + k^*} = \left[u_{eo} \frac{1 + k^*_{ep}}{1 + k^*} \right]$$

where k^* is the CEC retention factor, k^*_{ep} the velocity factor, t_m the migration time of the analyte and t_0 is the migration time of the EOF marker in the CEC column, k^* and k^*_{ep} are given by:

$$k^*_{ep} = \frac{u_{ep}}{u_{eo}}$$

$$k^* = \frac{[t_m(1 + k^*_{ep}) - t_0]}{t_0}$$

The plots of the natural logarithms of electrochromatographic retention factors against the reciprocal of absolute temperature are shown in Fig. 5B, in which non-linear data are shown.

The origin of non-linear Van't Hoff behavior is any reversible process which alters the enthalpy or entropy of adsorption [29]. These process may involve the analyte, the stationary, or the mobile phase. Dissociate processes such as ionization, changes in conformation, or changes in the extent to which the mobile phase interacts with either the analyte or stationary phase are examples of such reversible process. Additionally, the presence of multiple types of retention mechanisms or multiple types of binding sites may also lead to non-linear Van't Hoff plots. Therefore, the non-linear

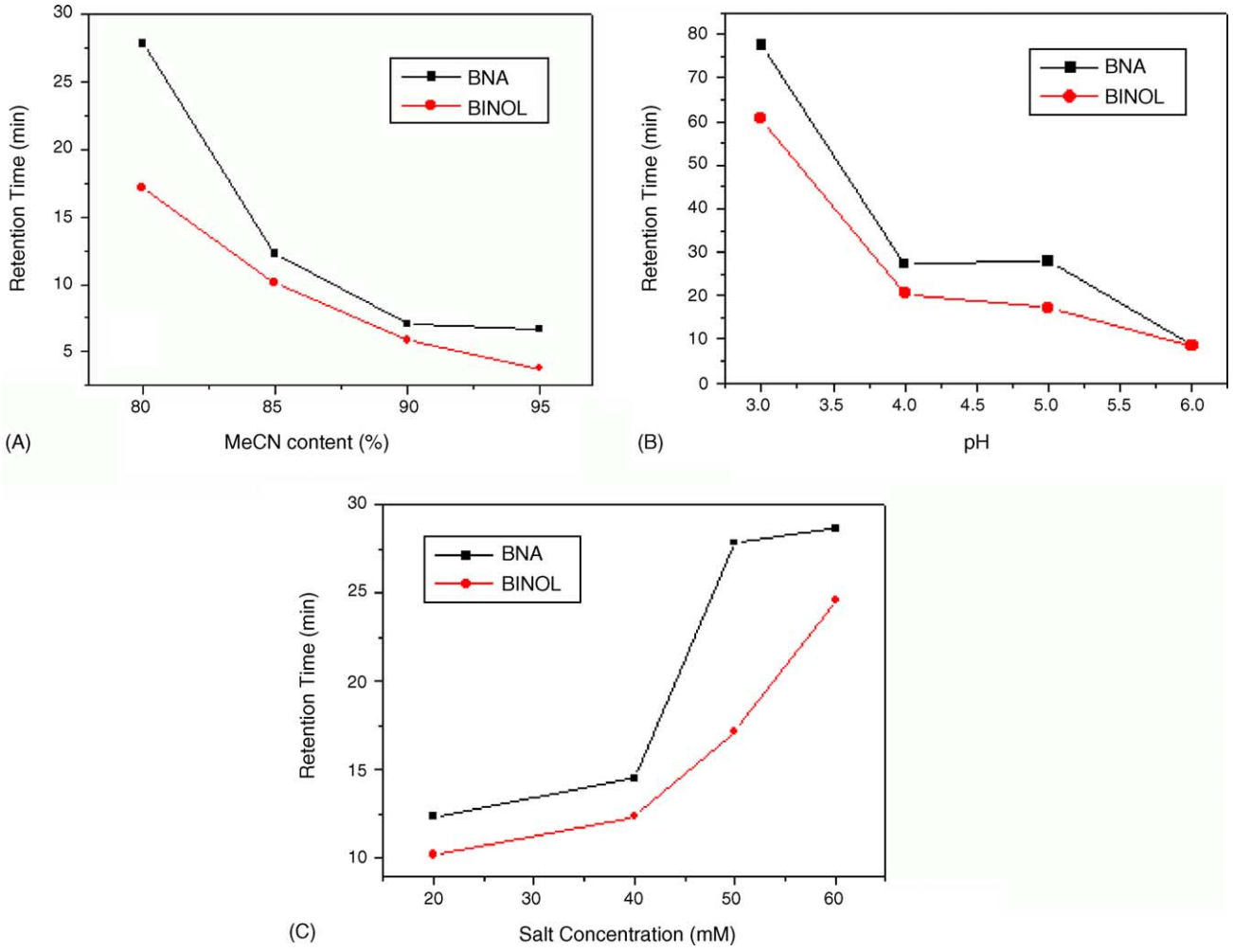

Fig. 4. Effect of CEC parameter on the retention time of BNA and BINOL on the MIP monolithic column. The capillary was thermostated to 25 °C and UV detection was carried out at 254 nm. Separation was performed on the MIP monolith at 15 kV and over-pressure of 20 psi. (A) Retention time against the acetonitrile concentration in the eluent. The electrolyte used was composed of acetonitrile (80–95%, v/v)/0.05 M acetate (pH 5.0) (20–5%, v/v). (B) Retention time against the pH value in the eluent. The electrolyte used was composed of acetonitrile/0.05 M acetate (pH 3.0–6.0) (80/20, v/v). (C) Retention time against the acetate concentration in the eluent. The electrolyte used was composed of acetonitrile/acetate (pH 5.0) (80/20, v/v) (0.02–0.06 M).

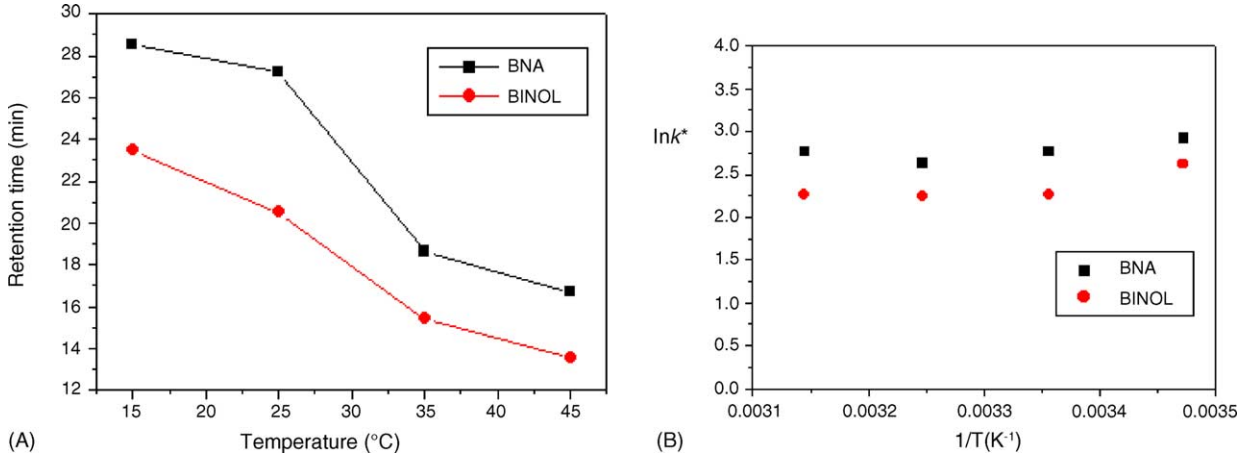

Fig. 5. Effect of temperature on (A) the retention and (B) Van't Hoff plot of BNA and BINOL on the MIP monolith. The column temperature was increased from 15 to 45 °C in 10 °C increments. CEC was performed at 15 kV and over-pressure of 20 psi. The electrolyte used was composed of acetonitrile/acetate (pH 4.0, 50 mM) (80/20, v/v). UV detection was carried out at 254 nm.

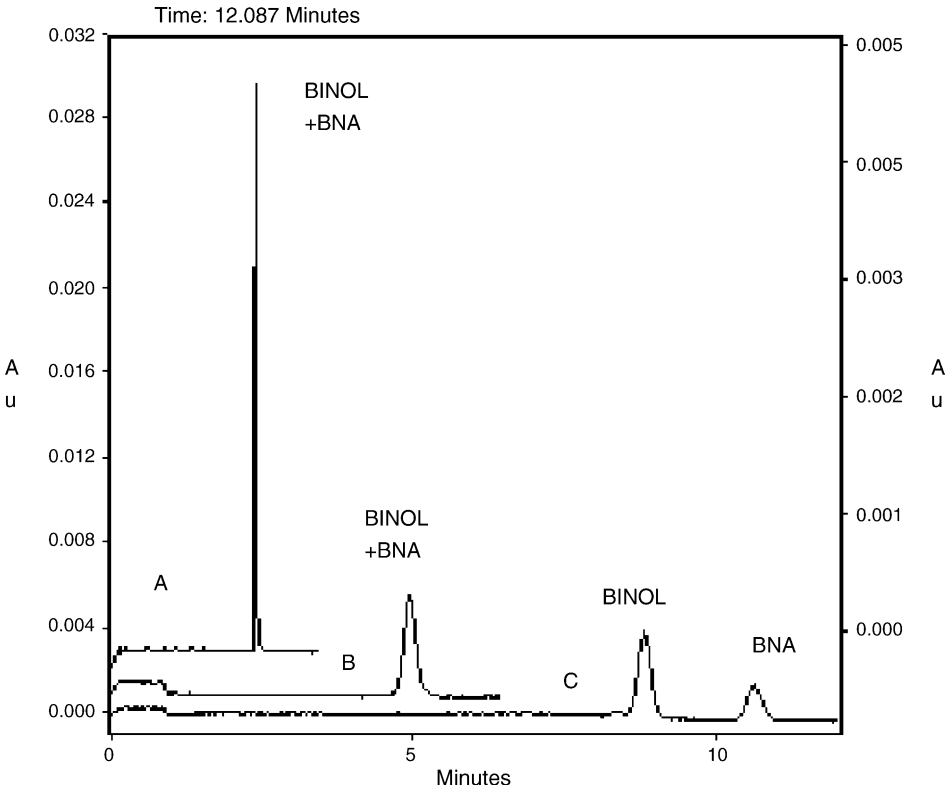

Fig. 6. Chromatograms of the separation of BNA and BINOL on the open column, blank monolithic column and monolithic MIP column, respectively. Separation was performed on the MIP monolith at 15 kV and over-pressure of 20 psi and the capillary was thermostated to 25 °C. The electrolyte used was composed of acetonitrile/50 mM acetate (pH 5.0) (80/20, v/v). UV detection was carried out at 254 nm. (A) Open column; (B) blank column; and (C) BNA-imprinted column.

Van't Hoff behavior observed here may be the presence of selective and non-selective binding site in the MIPs, as well as electrophoretic migration.

In summary, optimized condition of the separations for BINOL and BNA was performed on the MIP monolith at 15 kV and over-pressure of 20 psi and the capillary was thermostated to 25 °C. The electrolyte used was composed of acetonitrile/50 mM acetate (pH 5.0) (80/20, v/v).

3.4. Selectivity of the BNA-imprinted monolithic polymers

To test the function of molecular recognition of BNA-imprinted monolith, the separation of BNA and BINOL on MIP monolithic column, blank column and open column were shown in Fig. 6. Since the blank column was synthesized without templates, it did not possess recognition sites complementary to the spatial structure of BNA. As a result, from Fig. 6, BNA and BINOL cannot be separated on the blank column at all. In CE mode, a contribution to electrophoresis migration of BNA and BINOL was investigated and separation factor (α'), which measures the relative retention between HNA and BINOL is 1. On MIP monolith, retention of BNA varied dramatically and a base-line separation of BNA and BINOL was achieved. Separation factor (α') between BNA and BINOL is 1.62. The resolution (R_s) of BNA and BINOL is dramatically 4.33. This result contributes to high column efficiency obtained on BNA-imprinted monolithic column, i.e., theory plates of BNA was 43,000 plates/m (Fig. 6C). A R-BNA-imprinted monolithic column was prepared as same ratio of pre-polymerization except imprinted molecule, in which chiral separation was obtained, and imprinting effect not a convectional CEC was demonstrated on the MIP monolithic column.

Selectivity of the BNA-imprinted monolithic polymers toward BNA and its structurally related compound, BINOL, is examined. The difference between BNA and BINOL is functional group that can interact with the functional monomer to form a complex (the former is amide group and the latter is hydroxyl group) (see Fig. 1). The interaction between BINOL to MIPs is hydrogen bond, while the interaction between BNA and MIPs includes hydrogen bond and ion interaction. This means that the driving force of molecular recognition is mainly the strength of interaction of analytes to MIPs. Table 2 shows the separation factors for BNA and BINOL under the different CEC parameters.

The effect of the content of acetonitrile in the mobile phase on the selectivity of imprinted molecule was investigated. Increasing amounts of MeCN, a trend of the increased separation factors for BNA and BINOL is observed. Water in electrolyte can substitute for the protonated carboxyl of the polymer in forming hydrogen bonding with imprinted molecule,

Table 2
Separation factors of various CEC parameters of BNA and BINOL on the MIP monolith

Eluent	Separation factor
Effect of MeCN[a] (%)	
80	1.6
85	1.22
90	1.21
95	1.78
Effect of pH[b]	
3.0	1.28
4.0	1.33
5.0	1.62
6.0	–
Effect of salt[c] (M)	
0.02	1.21
0.04	1.18
0.05	1.61
0.06	1.16
Effect of temperature[d] (°C)	
15	1.22
25	1.33
35	1.21
45	1.23

[a] Eluent is as same as Fig. 4A.
[b] Eluent is as same as Fig. 4B.
[c] Eluent is as same as Fig. 4C.
[d] Eluent is as same as Fig. 5.

interfering directly with the binding of imprinted molecule on the surface of stationary phase. Therefore, because MeCN is a hydrophile solvent with a lower ability to form hydrogen bonds, high MeCN content surrounding has less influence on the binding between imprinted molecule and MIPs.

The effect of the concentration of salt in the mobile phase on the selectivity between BNA and BINOL is shown in Table 2. A trend of increased selectivity is observed with increasing salt concentration in the mobile phase. However, at a salt concentration 0.06 M, the selectivity decreases due to greater Joule heat in the higher salt concentration. Similar results has been observed in recent investigation of MIP open column [14].

Previously, Sellergren and Shea [22] showed that solute retention and selectivity on imprinted polymer follow a cation-exchange model. They also found that the separation factor was high at lower pH and decreased with increasing mobile phase pH value. It may be contributed to the increased nonselective sites with increasing mobile phase pH [30]. From Table 2, a trend of decrease in separation between BNA and BINOL is observed. It is interesting that at pH 5.0, maximum separation factor is obtained. At lower pH, BNA molecules are positively charged. Electrophoretic migration of BNA will play a important role and decrease the retention of BNA on the MIPs. At pH 6.0, almost no recognition of imprinting is observed. As a result, a compromise between imprinted effect and electrophoretic migration is obtained at pH 5.0.

According to Lin et al. [31], the selectivity of molecular recognition by molecular imprinting was temperature related and decreased with the increase of temperature, in which a linear Van't Hoff behavior was observed. They suggested that the interaction of analytes with the polymer is a diffusion-controlled process. In contrast, in the work of Sellergren and Shea [32], the selectivity increased with the increase of temperature. They suggested that the behavior is due to solute desolvation on formation of electrostatic interactions between the site and the solute. A compromise between thermodynamic and kinetic factors is observed in our investigation. The effect of temperature on the selectivity is shown in Table 2. No apparent difference of selectivity is observed under the different temperature except 25 °C. Similar results are also observed in S-naproxen-imprinted monolith [33].

4. Conclusion

A BNA-imprinted MIPs monolith in a capillary was prepared and evaluated in a CEC mode. An investigation of retention and separation of BNA and its structural analogue, BINOL, showed that CEC parameters affected the molecular recognition on the MIP. The major parameters included organic modifier concentration, pH value of buffer, salt concentration of buffer and column temperature. 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. Both thermodynamic and kinetic factors influence the molecular recognition on BNA-imprinted polymer. The findings here suggests that for a CEC-based MIP, the selectivity of molecular recognition is related to CEC parameter and optimum CEC condition has to be chosen. We believe that this fundamental study further contributes to the understanding of the processes of molecular recognition on MIPs in CEC mode.

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