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Preparation and Evaluation of a Molecularly Imprinted Polymer Derivatized Silica Monolithic Column for Capillary Electrochromatography and Capillary Liquid Chromatography

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A method for preparation of molecularly imprinted polymer (MIP) derivatized onto the surface of a monolithic silica capillary column was successfully developed. The vinyl groups were first introduced onto the silica monolith by immobilization of γ-methacryloxypropyltrimethoxysilane. Then the MIP coating was copolymerized and anchored onto the surface of the silica monolith. Acetonitrile was selected as porogen (solvent). The other preparation conditions, such as monomer concentration, temperature, and time of polymerization, were systematically studied. The obtained MIP-derivatized silica monolith using L-tetrahydropalmatine (L-THP) and (5*S*,11*S*)-(−)-Tröger's base (*S*-TB) as the imprinted template, respectively, was characterized in terms of the retention behavior of thiourea and toluene. Under the optimized CEC conditions, baseline enantioseparations of THP and TB were achieved in 4 min though the effective length of the columns was 8.5 cm. The result indicates that enough recognition sites were on the surface of silica monolith, resulting in strong recognition ability. Compared with a MIP organic monolith, the MIP-derivatized silica monolith exhibits better column efficiency and stability in CEC. Additionally, the comparison of these two kinds of monolithic columns was performed by capillary liquid chromatography. The separation on MIP-derivatized silica monolith was superior to that on the organic monolith.

The molecular imprinting technique has become a well-known means for the preparation of polymer material with high selectivity, which has been used in a variety of applications, such as separation media, artificial antibody mimics, and sensing devices. The resultant polymers can recognize the imprinted molecules and show substrate selectivity.[1−7] One of the most widely used applications of molecular imprinting polymers (MIPs) is as chiral stationary phases for separation of enantiomers by high-performance liquid chromatography (HPLC) and capillary electrochromatography (CEC).[8−13]

Traditionally, the MIP stationary phases have been prepared by bulk polymerization, suspension polymerization, multistep swelling polymerization, and precipitation polymerization methods. Despite the high yield for these methods, a large number of template molecules are needed in the preparation process, which is not cost-efficient. Since Matsui and co-workers[14] had employed an in situ polymerization technique to prepare MIP monolithic rods, this method has been used to synthesize the MIP in a stainless steel column or a capillary column without the tedious procedures of grinding, sieving, and column packing.[15−18] However, the obtained MIP monoliths for both HPLC and CEC often suffer from low efficiency because of the slow association/dissociation kinetics of the analytes with the MIP stationary phases.[12,19]

An alternative approach that may overcome this disadvantage is to prepare the MIP as a film coating onto the surface of

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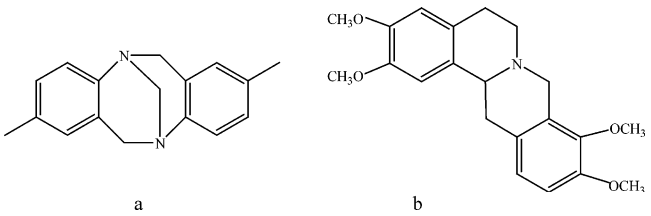

Figure 1. Molecular structure of (a) Tröger's base and (b) tetrahydropalmatine.

stationary phases. The MIP film coating at the capillary inner wall for open-tubular CEC (OT-CEC) has been reported.[20,21] The MIP coatings were covalently attached to the capillary by the activation of the capillary inner surface with vinyl groups. Schweitz[22] has synthesized MIP coatings in fused-silica capillary columns by use of a surface-coupled radical initiator. Huang et al.[23] also prepared an MIP film based on a 9-ethyladenine cavity for the recognition of nucleotide bases in OT-CEC. It is well known that the low sample capacity in OT-CEC is a major disadvantage because of low surface area. Quaglia et al.[24] packed MIP-silica composite beads into a capillary for enantioseparation of phenylalanine anilide in CEC. It is a problem to construct reproducible frits, which can lead to zone broadening and bubble formation.

Tanaka and co-workers[25,26] pioneered the fabrication of silica rods via a sol−gel process based on the hydrolytic polymerization of alkoxysilanes. To date, silica monoliths are widely applied for many fields of separation by HPLC and CEC.[25−27] Compared with the organic polymer monoliths, silica monoliths are not apt to swell or shrink in different organic mobile phases.[28] In the present study, the MIP film coating was anchored covalently onto the surface of silica monolith in capillary. The enantioseparations of tetrahydropalmatine (THP) and Tröger's base (TB) were successfully acquired by CEC and capillary liquid chromatography (cLC).

EXPERIMENTAL SECTION

Materials. DL-Tetrahydropalmatine (DL-THP) and L-tetrahydropalmatine (L-THP) were purchased from the National Institute for the Control of Pharmaceutical and Biological Products of China (Beijing, China). (5*S*,11*S*)-(−)-Tröger's base (*S*-TB) and (5*R*,11*R*)-(+)-Tröger's base (*R*-TB) were obtained from Fluka (Buchs, Switzerland). Their molecular structures are indicated in Figure 1. Ethylene dimethacrylate (EDMA) and trimethylolpropane trimethacrylate (TRIM) from Sigma (St. Louis, MO) were extracted with 10% aqueous sodium hydroxide and water and dried over anhydrous magnesium sulfate. Methacrylic acid (MAA) from Acros (Geel, Belgium) was distilled under vacuum. Tetramethoxysilane (TMOS) was from Chemical Factory of Wuhan University (Wuhan, China). Poly(ethylene glycol) (PEG, M_n = 10 000) was purchased from Aldrich (Milwaukee, WI). 2,2′-Azobisisobutyronitrile (AIBN) was obtained from Shanghai Chemical Plant (Shanghai, China) and recrystallized in ethanol before used. γ-Methacryloxypropyltrimethoxysilane (γ-MAPS) was purchased from Sigma. Fused-silica capillary with 75 μm i.d. × 375 μm o.d. was purchased from the Yongnian Optic Fiber Plant (Hebei, China). Water used in all experiments was doubly distilled and purified by a Milli-Q system (Millipore Inc., Milford, MA). HPLC-grade acetonitrile (ACN) was used for preparation of electrolyte. Other chemical reagents used were analytical grade.

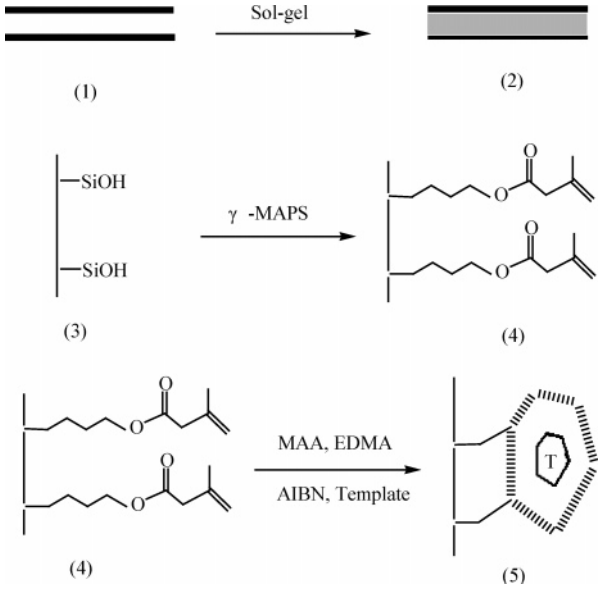

Figure 2. Schematic representation of preparation procedures of MIP film-derivatized silica monolithic column. (1) Open-tubular capillary; (2) silica monolith; (3) silanols on the surface of silica monolith; (4) vinylation on the surface of silica monolith; (5) formation of MIP film on the surface of silica monolith.

Preparation of MIP-Derivatized Silica Monolithic Column. The procedures for preparation of the MIP-derivatized monolithic column are illustrated in Figure 2.

Prior to the preparation of the silica monolith, the capillary was rinsed with 1.0 M NaOH for 30 min, water for 30 min, 1.0 M HCl for 2 h, and water for another 30 min, successively, and then dried in a GC oven at 110 °C for 12 h. The silica monolithic column was prepared according to the procedures previously reported.[29,30] Briefly, 1.06 g of PEG was dissolved in 10.0 mL of acetic acid solution (0.01 M). After 4.0 mL of TMOS was added, the whole solution was violently agitated in an ice bath for 45 min. The obtained sol was injected into the pretreated capillary and both ends were sealed, followed by immersing into a thermostat water bath at 40 °C for 24 h. The mesopores were tailored by treating the wet gel in 0.01 M ammonium hydroxide solution at 120 °C for 3 h. The capillary was then placed in a GC oven at 330 °C for 24 h to remove the organic moiety after evaporation of the solvent in capillary to dry of the gel.

The capillary silica monolith was rinsed with 0.1 M HCl solution for 1 h, and then with water until the pH value of the

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Table 1. Conditions for Preparation of L-THP Imprinted Polymer Derivatized Silica Monolithic Columns for Enantioseparation of Racemic THP

	amount (mmol)							
col	template	MAA	EDMA	TRIM	ACN (mL)	T (°C)	time (min)	permeability[a]
1	0.03	0.1	0.4	0	0.20	55	60	low
2	0.03	0.1	0.4	0	0.20	55	50	low
3	0.03	0.1	0.4	0	0.20	55	40	good
4	0.03	0.1	0.4	0	0.20	60	30	good
5	0.03	0.1	0.4	0	0.20	60	40	low
6	0.03	0.1	0.4	0	0.25	55	70	good
7	0.03	0.1	0.4	0	0.25	55	80	good
8	0.03	0.1	0.4	0	0.25	55	90	low
9	0.03	0.1	0.4	0	0.25	60	40	good
10	0.03	0.1	0.4	0	0.25	60	50	low
11	0.03	0.1	0	0.2	0.2	55	150	good
12	0.03	0.1	0	0.2	0.2	55	180	low

[a] Permeability represents whether the mobile phase could flow through the prepared monolithic columns.

outlet solution was 7.0. After subsequent flushing with methanol for 10 min, it was dried by passage of nitrogen gas in oven. The solution of γ-MAPS in methanol (1/1, v/v) was injected into the capillary with a syringe. It was then kept at 40 °C overnight after both ends were sealed with rubber. Finally, the capillary was rinsed with methanol and water successively to flush out the residual reagents and dried again.

The template (L-THP), functional monomer (MAA), cross-linker (EDMA), and initiator (AIBN) were dissolved in solvent (ACN) to form a homogeneous solution, in the compositions as indicated in Table 1, which was sonicated for 10 min and purged with dry nitrogen for 15 min to remove oxygen. Then the capillary was filled with the polymerization mixtures. After both ends of the capillary were plugged with silicon rubber, the polymerization was initiated thermally by immersing the capillary in a GC oven at 55 or 60 °C for a few minutes. Then the unreacted mixture was flushed out by a nitrogen stream with a pressure of 3 bar, and the remainder of the reagents were further polymerized and shrunk into a film coating at 55 or 60 °C. The prepared MIP-derivatized silica monolithic capillary column was washed with ACN/acetic acid (9/1, v/v) by an HPLC pump to remove the template molecule and unreacted monomers. Finally, the capillary with MIP stationary phase was equilibrated with the running buffer before use.

The *S*-TB imprinted polymer derivatized silica monolith was prepared according to the procedures described above.

Preparation of L-THP-Imprinted Organic Monolithic Capillary Column. The L-THP-imprinted organic monolithic column was also prepared according to the procedures previously reported.[31] In brief, the capillary was first immobilized with γ-MAPS to provide anchoring sites for the polymer and then dried after being rinsed with methanol and water successively to flush out the residual reagents. The template (L-THP), functional monomer (MAA), cross-linker (EDMA), and initiator (AIBN) were dissolved in porogenic solvents (toluene and dodecanol) to form a homogeneous solution. The solution was sonicated for 10 min and purged with dry nitrogen for 15 min to remove oxygen. After the capillary was full of the polymerization mixtures, both ends of the capillary were plugged with silicon rubber, and then the capillary was immersed in a water bath at 55 °C for 12 h. The obtained MIP monolithic capillary column was washed with ACN/acetic acid (9/1,v/v) by an HPLC pump to remove the unreacted reagents. Finally, the prepared polymer monolith was equilibrated with the running buffer prior to using.

Instruments and Methods. Electrochromatographic experiments were performed on an Agilent CE system (Hewlett-Packard, Waldbronn, Germany) equipped with a UV detector. Data were acquired and processed with ChemStation software. The electrolytes composed of ACN with varying concentrations of acetate buffer were filtered before use. For a silica-based monolithic column, a detection window was prepared by removing ~4 mm of the protecting polymer layer at 8.5 cm from one end of the capillary. For the organic monolithic column, a detection window was created by burning out a 2−3-mm segment of the outer polyimide coating at 8.5 cm from the outlet end of the capillary. The monolithic capillary column (total length, 33 cm) was placed in the instrument and equilibrated by applying a voltage of 10 kV until the baseline signal was stabilized. A pressure of 3 bar was applied to both inlet and outlet vials simultaneously if not otherwise stated. The samples were degassed by sonication and injected electrokinetically by applying a voltage of 5 kV for 4 s in CEC. The temperature was kept at 25 °C, and the detection wavelength was set at 214 nm. The resolution is calculated from the equation $R_s = 2(t_2 - t_1)/(w_1 + w_2)$, where t_1 and t_2 are the retention times of the first and second eluted enantiomers, respectively, and w_1 and w_2 are the baseline peak widths of the first and second eluted enantiomers, respectively.

cLC experiments were performed on an Agilent 1100 LC system (Hewlett-Packard) equipped with a micropump and a UV detector. The flow rate of pump was set at 60−200 μL/min. For obtaining a flow rate of nanoliters per minute, a T-union connector was used to serve as a splitter, with one end connected to the monolithic column and one end connected to a blank capillary (50-μm i.d.). The actual flow rate in the monolithic column was 100−400 nL/min, resulting in split ratios from 500:1 to 800:1. A 10-cm-long bare capillary was used to load the samples. After ~0.44 μL of sample solution was injected into this capillary, the capillary was connected between the micropump and T-union. So the sample was also split before injecting into column. The detection wavelength was set at 214 nm.

Scanning electronic microscopic (SEM) images were obtained by using a JEOL JSM-5600 scanning elctron microscope.

RESULTS AND DISCUSSION

Preparation of MIP-Derivatized Silica Monolithic Capillary Column. A silica monolithic capillary column has been successfully prepared and characterized previously.[29] The obtained silica monolith has the morphology of a continuous skeleton and large through-pores. For acquiring a stable MIP-derivatized onto silica monolith, vinylation of the surface of silica monolithic column must be performed by reaction of silanols and γ-MAPS. Thus, the MIP can be chemically anchored with the silica monolith by copolymerization.

In our case, two basic enantiomers, L-THP and *S*-TB, were selected as the imprinted molecules, respectively, which have been

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the model analytes for enantioseparation of THP and TB on an MIP organic monolith by CEC. Likewise, MAA and EDMA/TRIM were used as functional monomer and cross-linker, respectively. Although it was reported that ACN was not the best solvent (porogen) for synthesis of a MIP coating in an open-tubular capillary by Schweitz,[22] in the present work, ACN was still selected as the solvent/porogen because of its good solubility for a large number of polar template molecules and its wide use as a component of electrolyte buffers. As the selectivity and flow-through property of a monolithic MIP column are very important in CEC, preparation conditions such as the concentration of monomers, temperature, and time of polymerization need to be optimized.

A series of MIP-derivatized silica monolithic columns were prepared according to the conditions shown in Table 1. The results indicate that the polymerization temperature and time play very important roles in the preparation. The flow-through property of the columns prepared by polymerization at high temperature or long time would become low. Capillary column (col 1) was prepared with the polymerization time of 60 min at 55 °C. Even after applying a pressure of 20 MPa with an HPLC pump, the column was still in the occluded state and no solvent was flowing through the column. As the time of polymerization was decreased to 40 min, the remaining solution in the column (col 3) could be flushed out by a nitrogen stream. After then, this column would be used for CEC evaluation. However, further decreasing temperature or time of polymerization led to a reduction of the number of imprinting sites and resulted in a decrease of selectivity. The monolith (col 4) prepared by polymerizing at 60 °C for 30 min also exhibited a good flow-through property. However, both selectivity factor and resolution for THP obtained on col 4 by CEC were lower than those on col 3. The result can be explained by the low temperature of polymerization enhancing the imprinting. At low temperature, the complex compounds formed by functional monomer and template molecules in the prepolymerization solution can be more stable.[21]

The effect of amount of solvent (porogen) and another cross-linker, TRIM, on the preparation was also investigated. As indicated in Table 1, when the amount of ACN was increased, the column (col 7) could still have flow-through even though the polymerization time was extended to 80 min. As the cross-linker EDMA was substituted by TRIM, the polymerization time was extended to 150 min (col 11), but the recognition ability for L-THP was not increased as the selectivity factor and resolution obtained on col 11 were lower that those on col 3. Considering both preparation time and selectivity, the preparation conditions for col 3 were selected as the optimal conditions.

The relative standard deviation (RSD) for the EOF and retention times of analytes were less that 2.2% ($n = 3$), which meant good stability of the prepared MIP-derivatized silica monolithic column. Additionally, both column-to-column and batch-to-batch reproducibilities for the preparation of a monolithic column were also evaluated in term of the RSDs for the EOF and retention times of analytes, and the RSDs were less than 4.7 ($n = 3$) and 6.3% ($n = 3$), respectively. These results indicated that the prepared monolithic capillary could be easily reproduced.

Similarly, the *S*-TB imprinted polymer derivatized silica monolithic column was also obtained by changing the molar ratio of functional monomer and *S*-TB, as well as the polymerization temperature and time. The optimum preparation conditions were polymerized at 55 °C for 60 min by using 0.025 mmol of *S*-TB, 0.01 mmol of MAA, and 0.4mmol of EDMA dissolved in 0.20 mL of ACN.

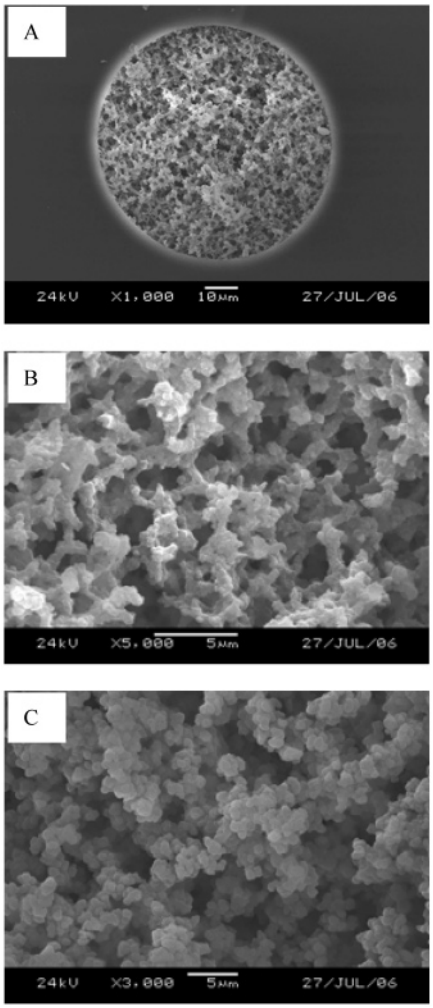

Figure 3. SEM photographs of (A) native silica monolith, (B) MIP-derivatized silica monolith, and (C) MIP organic monolith.

Characterization of MIP-Derivatized Silica Monolithic Column. Figure 3A illustrates the SEM photograph of native silica monolithic column with a diameter of 75 μm. It can be seen that the silica monolith has medium pores of ~2 μm and is well linked to the inner wall of the capillary.[29] The denser sol−gel skeleton not only decreases the mass-transfer resistance from mobile phase to stationary phase but also increases the surface area of monolithic material. Compared with the SEM photograph of the MIP-derivatized silica column shown in Figure 3B, the amount of mesopores and macropores was decreased, but the skeleton still existed. Because of the organic MIP derivatized onto the surface of the silica monolith, the image observed from the radial direction of the capillary with a microscope became obscure. Additionally, the morphology of this composite monolith is different from the particulate structure of an MIP organic monolith, whose SEM photograph can be seen in Figure 3C. The through-pores of the organic monolith were also obvious.

Figure 4A shows the migration times of thiourea and toluene on the vinyl-derivatized silica monolithic column under CEC by

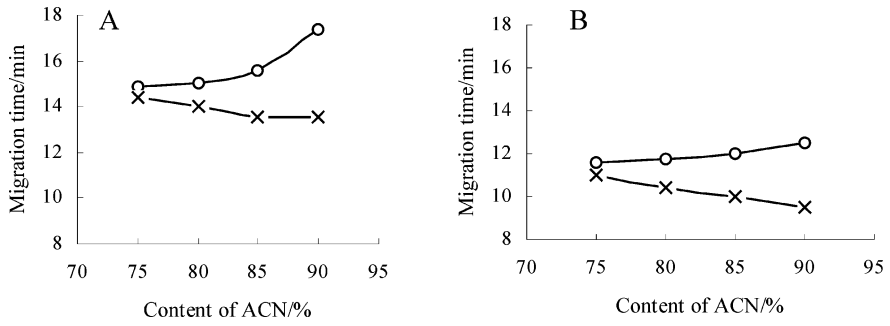

Figure 4. Effect of content of ACN on the migration times of thiourea (O) and toluene (×) on the (A) vinylated and (B) MIP film derivatized silica monolithic columns, respectively. CEC conditions: total length, 33 cm, effective length, 24.5 cm; mobile phase, ACN/5 mM HOAc buffer (pH 6.0); injection, 5 kV for 3 s; voltage, 10 kV; detection wavelength, 214 nm.

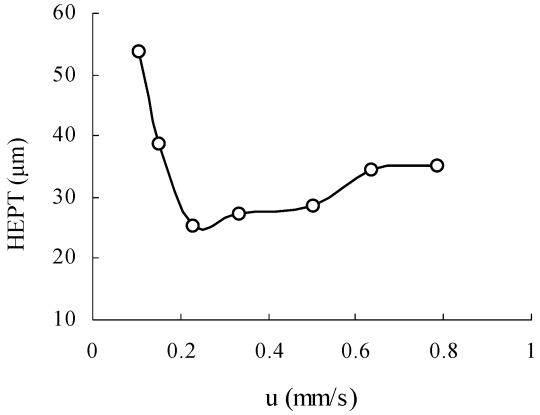

Figure 5. Plate height (HEPT) of retained thiourea vs linear velocity. CEC conditions are the same as in Figure 3 except that the applied voltages are from 4 to 25 kV.

using different running buffers containing different contents of ACN. It is found that the migration time of toluene decreased with an increase of ACN content ranging from 75 to 90%, while the migration time of thiourea increased with the increase of ACN content. Additionally, the migration times of thiourea were higher than those of toluene. These phenomena illustrated that the retention of thiourea is based on a normal-phase mechanism, which may be caused by the presence of a large number of naked silanols on the silica monolith although some vinyl groups were immobilized. As indicated in Figure 4B, similar migration behaviors for thiourea and toluene could be observed on the MIP-derivatized silica monolithic column, which means that the surface of the silica monolith is not completely covered by MIP film. By comparison of panels A and B in Figure 4, it can be easily seen that the migration mobilities of toluene and thiourea on the MIP-derivatized silica monolithic column were faster than those on the vinylated silica monolithic column. It can be ascribed to the higher EOF generated by both carboxyl groups and silanol groups on the surface of the MIP-derivatized silica monolithic column rather than the vinylated silica monolithic column, whose EOF was only generated by silanol groups.

Figure 5 indicates the relationship between the theoretical plate height (HEPT) and the velocity of retained thiourea. It can be seen that the lowest plate height is higher than 20 μm when the linear velocity was 0.23 mm/s. Compared with the column efficiency of other silica monolithic columns previously reported,[29] column efficiency of the obtained column in the present work is decreased remarkably, which may have resulted from the heterogeneous chemistry of the MIP film on the surface of the silica monolith.

Separation of Enantiomers on the MIP-Derivatized Silica Monolithic Column by CEC. As shown in Figure 6A, baseline enantioseparation of racemic THP is successfully acquired on the L-THP-imprinted composite monolithic column using ACN/5 mM acetate buffer (80/20, v/v, pH 6.0) as the running buffer by CEC, while the effective length of capillary column was only 8.5 cm. The running buffer was not further optimized.[31] It is indicated that the composite column has good selectivity and strong separation ability for THP. The L-THP-imprinted film was also synthesized onto the inner wall of an open capillary (effect length, 24.5 cm, total length, 33 cm × 75 μm i.d.) according to the preparation procedures reported by Tan and Remcho.[21,32] However, enantioseparation of THP was not acquired by OT-CEC. It is deduced that enough imprinting sites were immobilized onto the silica monolith as the surface area of the monolith is much bigger than that of the inner wall inside an open capillary. Furthermore, racemic TB is completely separated on the *S*-TB imprinted film derivatized silica monolith in 4 min (Figure 6B), while the effective length of the capillary column was 8.5 cm. This result further proves the property of a large surface area and enough recognition sites of this kind of composite monolithic column.

The L-THP-imprinted organic monolith was also prepared in a capillary based on the procedures previously reported by us.[31] Figure 6C illustrates the chiral separation of racemic THP on this organic monolith under the same electrochromatographic conditions as in Figure 6A. By comparison of Figure 6C and Figure 6A, it can be seen that the column efficiency obtained on the composite monolith is higher than that on the organic monolith, and the peak asymmetry factor of L-THP in Figure 6A is less than that in Figure 6C as the data show in Table 2. Furthermore, it is easily found that the baseline noise in Figure 6A is higher than that in Figure 6C. The noise signal obtained on the composite monolith is ~4 units, while on the organic monolith it is only 0.8 unit, which result in the lower detection sensitivity on the composite column than the organic monolith. The lifetime of the composite column and the organic column was compared, and it was found that the former was longer than the latter.

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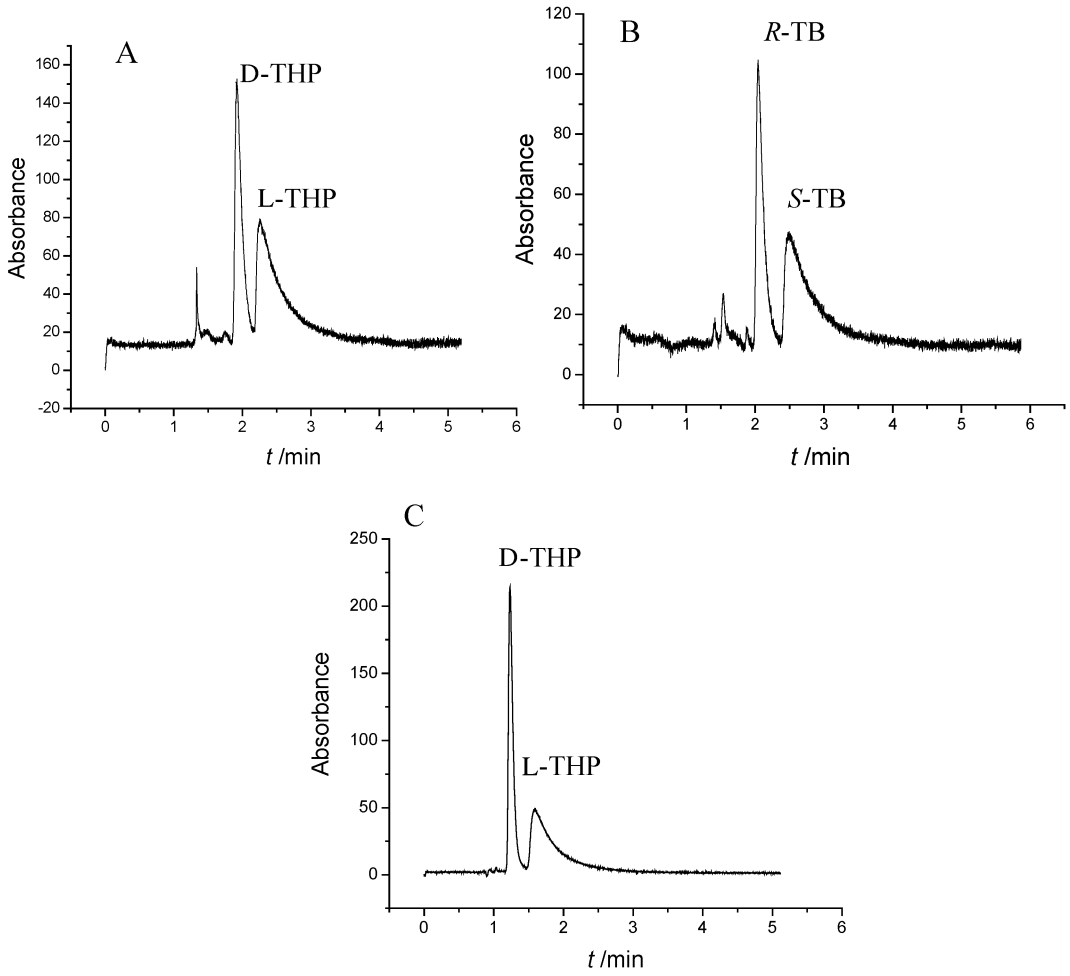

Figure 6. CEC enantioseparation of racemic THP and TB on (A) L-THP and (B) *S*-TB imprinted polymer derivatized silica monolithic columns, respectively, and (C) enantioseparation of racemic THP on L-THP imprinted organic monolithic column. CEC conditions: total length, 33 cm, effective length, 8.5 cm; mobile phase, (A) and (C) ACN/5 mM HOAc buffer (80/20, v/v, pH 6.0), (B) ACN/5 mM HOAc buffer (75/25, v/v, pH 6.0); injection, 5 kV for 3 s; voltage, 25 kV.

Table 2. Chromatographic Data Obtained on the L-THP Imprinted Polymer Derivatized Silica Monolith and Organic Monolith

column	μ_{EOF} (10^{-8} m^2 V^{-1} s^{-1})	$N_{D\text{-}THP}$ (m^{-1})	$N_{L\text{-}THP}$ (m^{-1})	R_s	As[a]
composite monolith	1.14	19 857	3993	1.08	5.8
organic monolith	1.73	17 263	1648	1.15	7.6

[a] As presents peak asymmetry factor of L-THP.

For solving the problem of low detection sensitivity, a zero dead volume union was used to connect a 20-cm-long composite monolith with a 13-cm-long blank capillary, and the detection window was prepared by removing ~4 mm of the protecting polymer layer at 8.5 cm from one end of the blank capillary. The effect of the joint on the separation was evaluated. As can be seen in Figure 7A, the enantioseparation of THP was also baseline separated on the segmented capillary column in spite of the significant loss of efficiency. The signal noise is lower than that shown in Figure 7B; however, it seems that the detection sensitivity was not increases because of the peak broadening. It is anticipated that the problem may be resolved by using a transparent capillary and UV-initiated polymerization; thus, the detection window can be prepared by removing ~0.5 cm of the outer protecting layer and then covering to prevent polymerization in this area of the capillary.

The effect of applied voltage on the chiral separation was investigated on the L-THP imprinted polymer derivatized silica monolithic column. As indicated in Table 3, the separation time is greatly shortened from 15 to 3 min with an increase of applied voltage from 5 to 25 kV, and the resolution of THP is gradually decreased. The effect of sample loading on separation was studied by electrokinetic injection with application of different voltage and time, and the obtained results are illustrated in Table 4. As the sample injection increased, the retention time of analyte decreased, and the resolution reduced sharply. These phenomena are similar to that in HPLC and may be due to the limited number of recognition sites on the surface of the monolith.[33,34]

Enantioseparation of THP on the MIP-Derivatized Silica Monolithic Column by cLC. As a promising alternative of a miniaturized separation technique to CEC, cLC has become very

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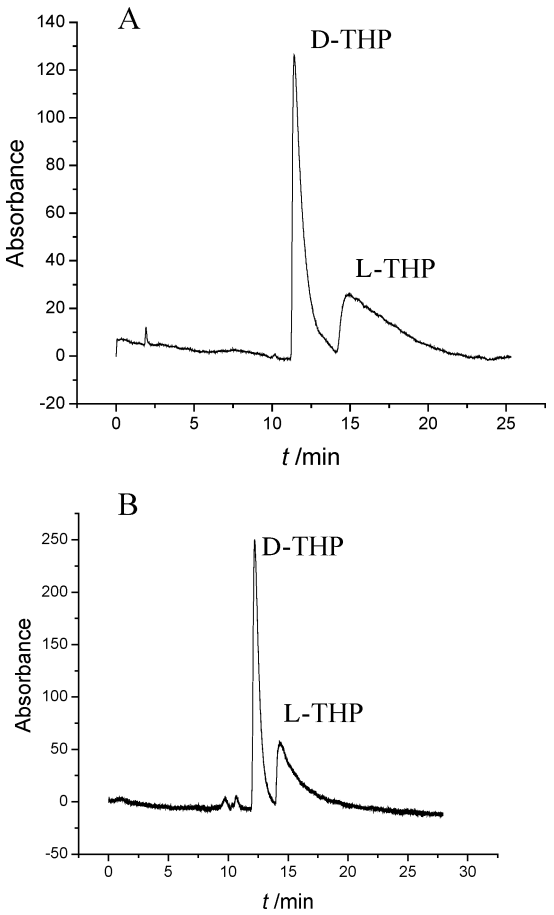

Figure 7. Enantioseparation of racemic THP on L-THP-imprinted composite monolithic column by CEC. CEC conditions: column, (A) composite monolithic column (20 cm) hyphenated with an open-tubular capillary (13 cm), (B) composite monolithic column (33 cm), effective length, 24.5 cm; mobile phase, ACN/5 mM HOAc buffer (80/20, v/v, pH 6.0); injection, 5 kV for 5 s; voltage, 10 kV.

Table 3. Effect of the Applied Voltage on the Retention and Resolution of Racemic THP on the L-THP Imprinted Polymer Derivatized Silica Monolithic Column[a]

voltage/kV	t_D/min	t_L/min	R_s
5	10.56	12.74	1.32
10	4.92	5.80	1.25
15	3.25	3.82	1.18
20	2.47	2.92	1.12
25	1.92	2.27	1.08

[a] CEC conditions: total length, 33 cm; effective length, 8.5 cm; mobile phase, ACN/5 mM HOAc buffer (80/20, v/v, pH 6.0); injection, 5 kV for 3 s; applied voltages from 5 to 25 kV.

Table 4. Effect of Sample Loading on the Retention of Resolution of THP Enantiomers on the L-THP Imprinted Polymer Derivatized Silica Monolithic Column[a]

injection	t_D/min	t_L/min	R_s
10 kV × 8 s	4.36	4.72	0.85
10 kV × 6 s	4.52	5.13	0.96
5 kV × 6 s	4.78	5.56	1.12
5 kV × 3 s	4.92	5.80	1.25

[a] CEC conditions: total length, 33 cm; effective length, 8.5 cm; mobile phase, ACN/5 mM HOAc buffer (80/20, v/v, pH 6.0); voltage, 10 kV.

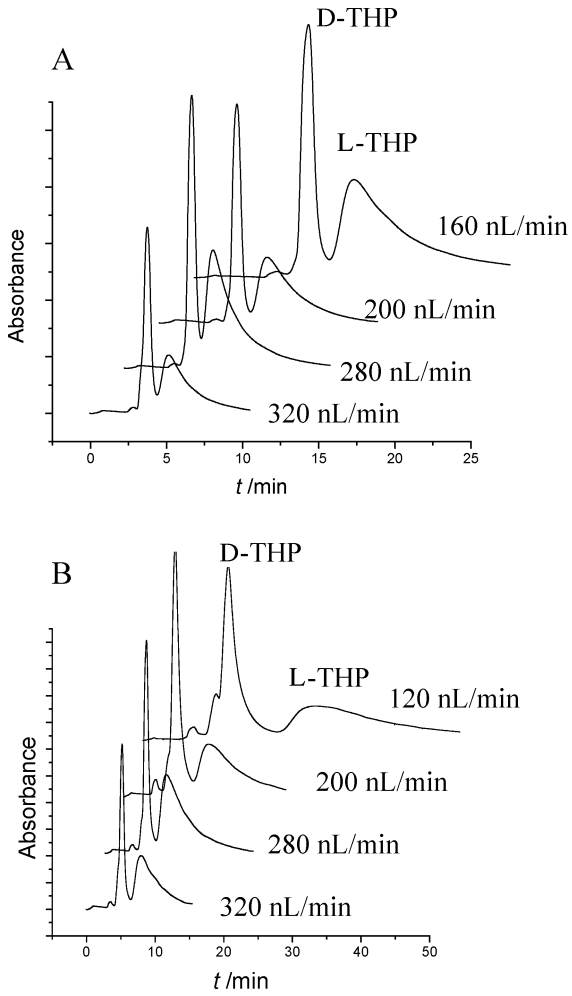

Figure 8. Enantioseparation of racemic THP on L-THP imprinted (A) polymer derivatized monolithic column and (B) organic monolith, respectively, by cLC at different flow rates. Chromatographic conditions: column length, 25 cm; mobile phase, ACN/5 mM HOAc buffer (80/20, v/v, pH 6.0); injection, 0.44 μL; detection wavelength, 214 nm.

attractive because of low consumption of chemicals, excellent selectivity, and high column efficiency. In this case, the L-THP-imprinted composite monolith prepared was exploited for chiral separation by cLC. Figure 8A indicates enantioseparation of THP at different flow rates when using ACN/5 mM acetate buffer (80/20, v/v, pH 6.0) as the mobile phase, which has been used in CEC separation. Although the composition of mobile phase was not further optimized, racemic THP could be well enantioseparated. The resolution is slightly decreased with an increase of the flow rate in the range of 160−320 nL/min, while the separation time is shortened from 20 to 10 min. This ascribes to the slow mass transfer of the imprinted analytes on the MIP. The retention time of D-THP is very close to the dead time, suggesting that the interaction between nonimprinted analyte and stationary phase is very weak. In addition, it is found that the column efficiency is lower than that obtained by CEC. However, the mode of gradient elution can be easily performed in cLC as similar to conventional LC to improve the peak sharp of enantiomer.[17]

To compare separation ability, the L-THP-imprinted organic monolith was also evaluated in cLC. The results are exhibited in Figure 8B. Under the same chromatographic conditions, enantioseparation of THP was acquired at the flow rate of 120−320 nL/min. The separation time dramatically decreases from 45 to 15 min as the flow rate increases, while the resolution is only slightly reduced. The retention of both D-THP and L-THP on the organic column is stronger than those on the composite column, which proves that the number of recognition sites on the surface of the silica monolith is smaller than that on the organic monolith. However, as far as the column efficiency and separation time are concerned, the former is superior to the latter. The effect of sample loading on the separation was also investigated on these two kinds of monolithic columns. The results show that both resolution and retention time on a composite monolith and organic monolith are gradually decreased with an increase of the sample loading. This phenomenon can be also observed in the conventional LC separation.[35]

CONCLUSIONS

A method of preparation of a molecularly imprinted film derivatized silica monolithic column for chiral separation in CEC and cLC was presented. The silica monolith confined in a capillary was first prepared by a sol−gel process, and then the MIP film was derivatized onto the surface of the silica monolith with a copolymerization reaction. Although the surface of the silica monolith was not completely covered by the MIP film, this kind of MIP-derivatized silica monolith still exhibited good selectivity and strong recognition ability. Two chiral compounds, THP and TB, in this case, were baseline separated by CEC within 4 min. In comparison with the previous method for preparation of an MIP organic monolith or film for CEC or OT-CEC, respectively, this method has some advantages such as large surface area and good stability. Additionally, ACN or another polar solvent can be used as the solvent (porogen); thus, it will extend the application area for preparation of a large number of polar molecules imprinted polymer derivatized onto the silica monolith. By comparison of the obtained composite monolith and organic monolith, it is found that the former exhibits stronger recognition ability, better peak symmetry, and higher column efficiency in both CEC and cLC.

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