Journal of Chromatography B, 875 (2008) 317–322

Contents lists available at ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/chromb

The covalently bonded cellulose tris(3,5-dimethylphenylcarbamate) on a silica monolithic capillary column for enantioseparation in capillary electrochromatography☆

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ARTICLE INFO

Article history:
Received 21 March 2008
Accepted 11 May 2008
Available online 16 May 2008

Keywords:
Capillary electrochromatography
Cellulose
tris(3,5-dimethylphenylcarbamate)
Covalently bonded chiral stationary phase
Enantioseparation
Silica monolithic column

ABSTRACT

A chiral capillary monolithic column for capillary electrochromatography (CEC) was prepared by covalent bonding of cellulose tris(3,5-dimethylphenylcarbamate) (CDMPC) on the silica monolithic matrix within the confine of a 50-μm i.d. bare fused silica capillary. Several pairs of enantiomers including neutral and basic analytes were baseline resolved on the newly prepared chiral capillary monolithic column in CEC with aqueous mobile phases. Fast enantioseparation was achieved due to the favorable dynamic properties of silica monolith. The covalent bonding of CDMPC as the chiral stationary phase for CEC also enabled the use of THF in mobile phase for enantioseparation of prazquantel by overcoming the incompatibility of THF and the physically coated CDMPC on a column.

1. Introduction

Capillary electrochromatography (CEC) has attracted increasing interest as it combines the high selectivity of HPLC and the high efficiency of CE. In addition, as a miniaturized separation technique, it shows a number of advantages such as the low consumption of stationary phases and solvents, need of smaller samples, environmental safety, and easy coupling to mass spectrometry, etc. Traditionally, enantiomer-selective stationary phases or chiral selectors used in HPLC have been transferred to CEC, resulting in the current use of three column technologies: open-tubular, conventionally particulates packed, and monolithic columns.

For open-tubular columns, the chiral selectors [1] are coated or immobilized on the inner surface of the capillaries, thus there are no packing materials in the capillaries and no frits are needed. These columns suffer from low phase ratio, ease of overloading, and low sensitivity in UV detection. In particulates packed column, the CSPs were packed into the capillaries and restricted by the retaining frits, such as CD and its derivatives [2–4], protein [5], macrocyclic antibiotic [6], chiral polyacrylamides [7], and Pirkle-type CSP [8]. These columns have a high-phase ratio and high enantioselectivity, but the bubble formation and poor repeatability caused by the supporting frits retarded the application of particulate packed column in CEC for the practical routine analysis of enantiomers. The problems encountered in these columns led to the development of a new concept of packed CEC columns, i.e., monolithic columns. It can be prepared by *in situ* polymerization within the confines of capillaries, thus avoiding the end-frits which required for particulate columns to secure the packing materials in place [9]. Chiral stationary phases based on the monoliths for CEC have been prepared by immobilization of diverse chiral selectors such as proteins [10–12], cyclodextrins [13–15], ligand-exchange type phase [16], macrocyclic antibiotics [17–20], crown ethers [17], and "Pirkle"-selector [21] onto the silica or polymer-based rods.

Polysaccharide derivative CSPs have been widely applied in HPLC [22] with good chiral selectivity. In CEC, polysaccharide derivatives including cellulose tris(3,5-dimethylphenylcarbamate)

Abbreviations: CBMPC, cellulose 2,3-bis(3,5-dimethylphenylcarbamate); CDMPC, cellulose tris(3,5-dimethylphenylcarbamate); CSP, chiral stationary phase; MPM, mobile phase modifier; THF, tetrahydrofuran.

☆ This paper is part of the special issue 'Enantioseparations', dedicated to W. Lindner, edited by B. Chankvetadze and E. Francotte.

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1570-0232/$ – see front matter © 2008 Published by Elsevier B.V.
doi:10.1016/j.jchromb.2008.05.019

(CDMPC) [7,23–26], cellulose tris(4-methylbenzoate) [27,28], cellulose tris(3,5-dichlorophenylcarbamate) [29–31], and amylose tris(3,5-dimethylphenylcarbamate) [27,28] have also been used for the enantioseparation by coating or bonding on silica-based particulate packed columns or monolithic column. For polysaccharide derivatives coated column, some solvents, such as THF or chloroform, could swell or dissolve the polysaccharide derivatives. Thus, these solvents could not be used as components of the mobile phase [32]. This restriction could be overcome by the development of chemically bonded CSPs [24,25,33], and it was observed that the separation of some enantiomers could benefit from the adoption of THF in the mobile phase [25]. Interestingly, no attention has been paid to the covalently bonded polysaccharide derivative-based capillary monolithic CSPs for CEC, although a promising application of such kinds of columns was reported by Chankvetadze et al. [34] for HPLC. In CLC, silica monoliths modified with polysaccharide derivatives have been used for the enantioseparation. Amylose tris(3,5-dimethylphenylcarbamate) (ADMPC) [35] and CDMPC [36] have both been successfully coated on to silica monoliths by Chankvetadze et al. Crosslinked amylose and cellulose derivatives were also immobilized on to silica monoliths via radical polymerization [37] in their group.

In our work, a bonded-type of CDMPC CSP was synthesized for enantioseparation in CEC. Enantiomer separations by CEC using the prepared CSP were performed under aqueous mobile phases. Particularly, the advantages of the broadened choice of solvents offered by the bonded CSP were discussed.

2. Experimental

2.1. Chemicals and reagents

Tetramethoxysilane (TMOS) was obtained from Chemical Factory of Wuhan University (Wuhan, China). Poly-(ethylene glycol) (PEG, MW = 10,000) was purchased from Aldrich (Milwaukee, WI, USA). 3-Glycidoxypropyl trimethoxysilane (3-GPTS) was purchased from Acros (NJ, USA). Microcrystalline cellulose and acetonitrile (ACN) were purchased from Merck (Darmstadt, Germany). Trityl chloride, 3,5-dimethylphenyl isocyanate, boron trifluoride etherate, racemic benzoin, indapamide, praziquantel, Tröger's base, and pindolol were obtained from Sigma–Aldrich (Gillingham, Dorset, UK). Methanol (HPLC grade) was obtained from Yuwang Chemical Company (Shandong, China). Water was purified by a Milli-Q system (Millipore, Milford, MA, USA). Cellulose 2,3-bis(3,5-dimethylphenylcarbamate) (CBMPC) was home made as described in literature [34].

2.2. Preparation of chiral silica monolithic column with covalently bonded CDMPC

2.2.1. Preparation of silica monolithic capillary column

The bare fused silica capillary (50 μm i.d. × 365 μm o.d.) was purchased from Yongnian Optic Fiber Plant (Hebei, China), and used to prepare the silica monolithic capillary columns using the sol–gel process as described in the literature [38]. After the synthesis of the silica monolith within the confine of a capillary, an *in situ* re-hydroxylation process was carried out to maximally generate the silanol groups on the surface of the silica monolithic matrix. Namely, a 1-M HCl solution was continuously pumped through the synthesized monolithic matrix within a capillary for 3 h at room temperature. And then, the column was flushed with water until the pH of effluent was ca. 7. Followed by a subsequent methanol rinse, the silica monolithic capillary column was purged with nitrogen overnight to dryness. Lastly, both ends of the prepared column were capped with two GC septa for further modification.

2.2.2. In situ covalent bonding of CDMPC onto a silica monolith

The *in situ* covalent bonding of CDMPC onto the prepared silica monolithic capillary column was carried out with the approach described by Chankvetadze et al. [34] with appropriate modification. Briefly, 20 μL of 3-GPTS was firstly dissolved in 200 μL of toluene, which was then pumped into a pre-prepared silica monolithic capillary column for introducing the epoxy groups onto the silica monolith by a 10-h reaction at 110 °C. After the completion of the reaction, the 3-GPTS modified silica monolithic column was respectively rinsed by toluene and methanol, and dried by a stream of nitrogen at room temperature for 3 h.

Then, the afore-dried 3-GPTS modified silica monolithic column was filled with a solution of 60 mg/mL CBMPC in acetone by a 10-MPa pumping pressure as described in the literature [26]. After the complete filling of CBMPC solution, the monolithic capillary column was removed from the pumping platform, and first left to dry at ambient pressure and temperature for 7 days and then was further dried in a vacuum oven at 40 °C overnight. At last, an on-line detection window was created by removing ~5 mm of polyimide coating at an appropriate position outside the capillary column. The obtained CBMPC-coated silica monolithic capillary column as CSP(1) was evaluated for enantioseparation in CEC.

For the preparation of CDMPC covalently bonded silica monolithic capillary column, a pre-prepared CBMPC-coated silica monolithic capillary column was filled with a solution of 10% (v/v) BF_3 etherate in dry toluene, and sealed with two GC septa to avoid the evaporation of the reagent from the column and left overnight at room temperature. After the above treatment, the column was then flushed with acetone to remove the unreacted cellulose derivative and dried in vacuum oven at 40 °C for 12 h. Moreover, the prepared CDMPC silica monolith capillary column was filled with a solution of 15 μL 3,5-dimethylphenylisocyanate in 35 μL of pyridine, and reacted at 80 °C for 12 h. And, subsequently, the column was respectively washed by pyridine and methanol to remove the unreacted isocyanate and finally dried at 80 °C for 12 h in vacuum oven. The final CDMPC-bonded silica monolithic capillary column as CSP(2) was also evaluated in CEC for enantioseparation.

2.3. Separation conditions in CEC

The enantioseparation in CEC was carried out on a CE instrument-P/ACE™ MDQ System (Beckman, Fullerton, CA, USA) equipped with a UV diode-array detector, while the temperature for column was set at 25 °C and wavelength for detection was set at 214 nm. All analytes were dissolved in running mobile phase to give final concentrations ranged from 0.5 to 1.0 mg/mL. Electrokinetic injection was adopted to load samples by applying 10 kV voltage for 1 s. The chiral resolution factor (R_s) were calculated from the equation $R_s = 2[t_{R2} - t_{R1}]/[W_2 + W_1]$, where t_{R1} and t_{R2} are the retention times of the first and second eluted enantiomers, respectively, while W_1 and W_2 are the peak widths of these two enantiomers in elution order.

3. Results and discussion

3.1. Characteristics of CDMPC-bonded silica monolith

Fig. 1 shows the SEM photographs of the monolithic silica rod functionalized with CDMPC. It can be seen that the monolithic rod has morphology of continuous skeleton and large through-pores, and the monolithic rod is tightly bonded to the inner wall of capillary column. The large through-pores diameter is around 2 μm.

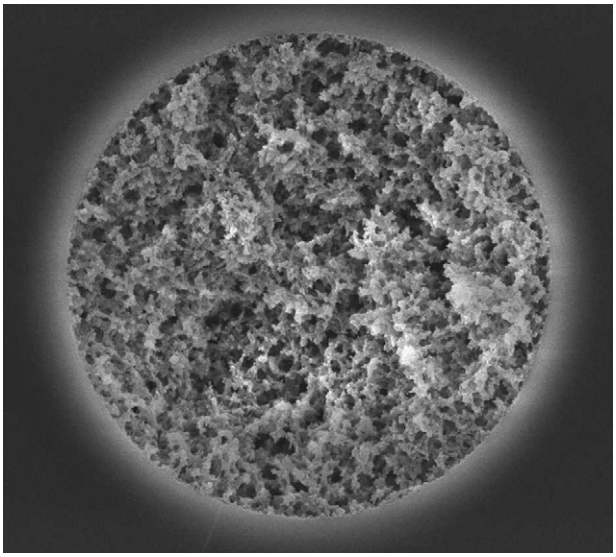

Fig. 1. SEM photographs of monolithic silica inside a capillary at magnifications of 1500×.

These characters lead to favorable dynamic properties of the monolithic silica capillary columns, and no frits are needed to retain the stationary phase. The permeability changed little after modified with CDMPC, which suggested that the covalent bonding process had little effect on the dynamic property of the column.

3.2. Enantioseparation on CBMPC-coated monolithic column

For finally obtaining the CDMPC-bonded chiral stationary phases on the silica monolithic capillary column, the pre-coating of CBMPC on the silica monolith as the intermediate stationary phase was necessary for further conversion from CBMPC to CDMPC catalyzed with BF_3 etherate. Also, as mentioned by Chankvetadze et al. [34], the CBMPC could provide good enantiomeric resolving performance as well. Thus, the CBMPC-coated chiral stationary phase (CSP1) on a silica monolithic matrix as the intermediate product of the final CSP2 was first evaluated in CEC for the confirmation of the successful coating of cellulose derivatives.

Because the electroosmotic flow (EOF) generated on the silica monolithic column was from anode to cathode and also the CDMPC chiral stationary phase showed good resolution performance to racemates [32], the basic (tetrahydropalmatine) and neutral (Tröger's base) racemates were selected as the standard analytes for testing the enantioseparation capability of the CSP1 column under aqueous mobile phases containing 40% acetonitrile and phosphate buffer or triethylamine phosphate buffer at pH 6.8. Typical electrochromatograms were presented in Fig. 2a and b for tetrahydropalmatine and Tröger's base, respectively. It can be seen that, two pairs of enantiomers as basic (tetrahydropalmatine) and neutral (Tröger's base) analytes were successfully baseline resolved with these aqueous mobile phases. Because the basic enantiomers (tetrahydropalmatine) were positively charged under this pH value, a competing base (TEA) was added to the mobile phase to weaken the peak tailing caused from the electrostatic interaction between the analytes and partially dissociated hydroxyl groups on the silica monolith. The obtained R_s values for tetrahydropalmatine and Tröger's base were 3.36 and 1.3, respectively. This result confirmed that the coating of the cellulose derivative, CBMPC, on the silica monolithic capillary column was successful, which thus ensured the further covalently bonding of CDMPC on silica monolithic matrix.

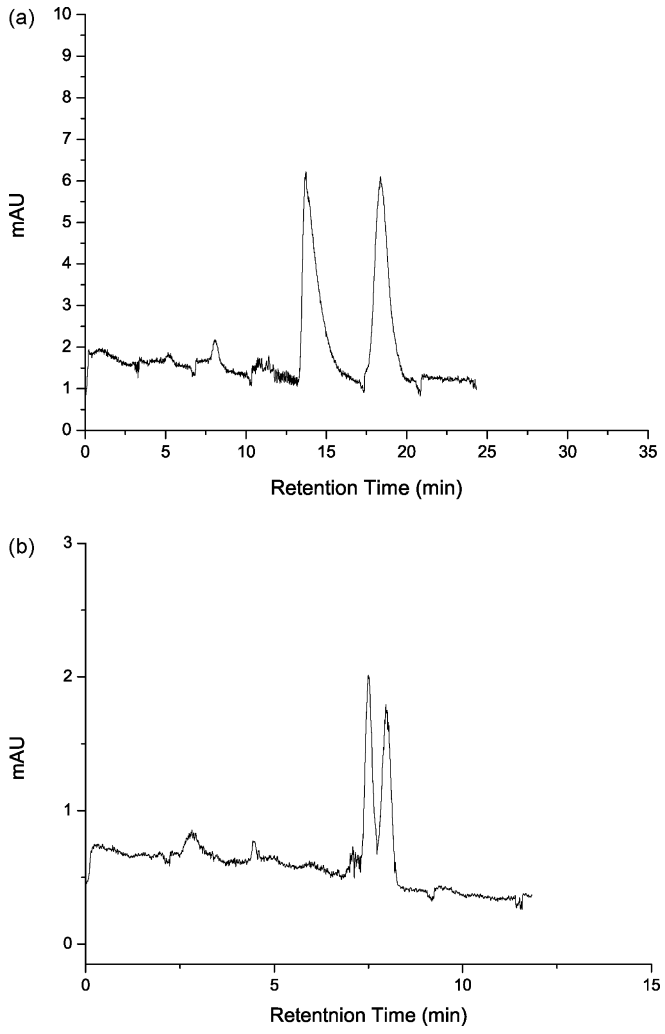

Fig. 2. Enantioseparation on CBMPC-coated silica monolithic capillary column. Experimental conditions: column, 50 μm i.d. silica monolithic capillary column coated with 50 mg/mL of CBMPC in acetone; solutes: (a) tetrahydropalmatine; (b) Tröger's base; other conditions were the same as in Table 1.

3.3. Enantioseparation on CDMPC-bonded monolithic column

After the treatment of CBMPC-coated silica monolithic capillary column with BF_3 etherate, the CDMPC was finally covalently bonded onto the silica monolithic matrix within the confine of the capillary column. For investigating the chiral resolving performance of the finally obtained CDMPC-bonded stationary phases (CSP2) in CEC, six pairs of basic and neutral enantiomers were tested using aqueous mobile phases, which contained 40% acetonitrile and phosphate buffers. The obtained electrochromatographic results were listed in Table 1. Most of enantiomers were successfully resolved with the enantiomeric resolution factors (R_s) ranged from 0.58 to 3.07. In Table 1, four pairs of neutral enantiomers were tested with a neutral mobile phase, and three of them were successfully resolved. Typical electrochromatograms of Tröger's base and indapamide were presented in Fig. 3b and d. Here, one thing has to be mentioned is that the detection sensitivity for analytes on the polysaccharide derivative-bonded silica monolithic column was impaired. This probably resulted from the absorption of UV light by either the silica matrix or the bonded cellulose derivatives on silica. Nevertheless, the enantioseparation on the prepared CSP silica monolithic column still could be realized in CEC with an acceptable response in this work.

Table 1
Electrochromatographic data obtained on the CDMPC-bonded silica monolithic capillary column.

Racemates	Mobile phase	t_1 (min)	t_2 (min)	R_s	N_1 (plates/m)	N_2 (plates/m)
Tröger's base	b	7.67	9.07	3.07	7,100	8,800
Tetrahydropalmatine	a	7.80	9.45	2.12	9,700	12,700
Pindolol	a	5.55	5.98	1.81	42,700	50,540
Indapamide	b	8.09	9.05	1.55	14,000	14,200
Benzoin	b	6.35	7.29	1.38	7,100	9,300
Prazquantel	b	9.73	10.20	0.58	20,100	16,400

Experimental conditions: column, 50 μm i.d. silica monolithic capillary column with covalently bonded CDMPC; mobile phase, (a) 4 mM triethylamine phosphate buffer containing 40% ACN at pH 6.8; (b) 4 mM phosphate buffer containing 40% ACN at pH 6.8; applied voltage, 10 kV; injection, 10 kV × 1 s; total length of capillary column, 31 cm, effective length, 20 cm.

The other two pairs of basic racemates (tetrahydropalmatine and pindolol) were also successfully resolved as shown in Fig. 3a and c by using TEA as a competitive additive in mobile phase. By comparing to the results obtained on CSP1 and CSP2, the R_s value of tetrahydropalmatine was decreased from 3.36 on CSP1 to 2.12 on CSP2, while the R_s value of Tröger's base was increased from 1.3 on CSP1 to 3.07 on CSP2. These results indicated that the covalent bonding of cellulose derivative on a silica monolithic capillary column as chiral stationary phase was successful in this work for CEC. However, due to the modification of hydroxyl groups of cellulose derivative and other unclear change of the cellulose derivative during the covalent bonding, the enantiomeric resolving performance was thus slightly differed from the coated cellulose derivative.

Recently, fast enantiomer separations are becoming more promising because the modern synthesis, such as combinatorial chemistry, can generate numbers of enantiomers in short time. Increasing the mobile phase flow rate is a direct way to shorten the analysis time. Thereafter, we tried to realize faster enantioseparation via applying high voltage. Fig. 4 shows the electrochromatogram for fast separation of indapamide. It can be seen that the enantiomers were separated in an even shorter time, only about 4 min. The highly effective enantioseaparation could be attributed to the favorable dynamic properties of silica monolith and strong driving force offered by CEC.

The enantioseparation on the silica monolithic column coated with polysaccharide derivatives including ADMPC [35] and CDMPC

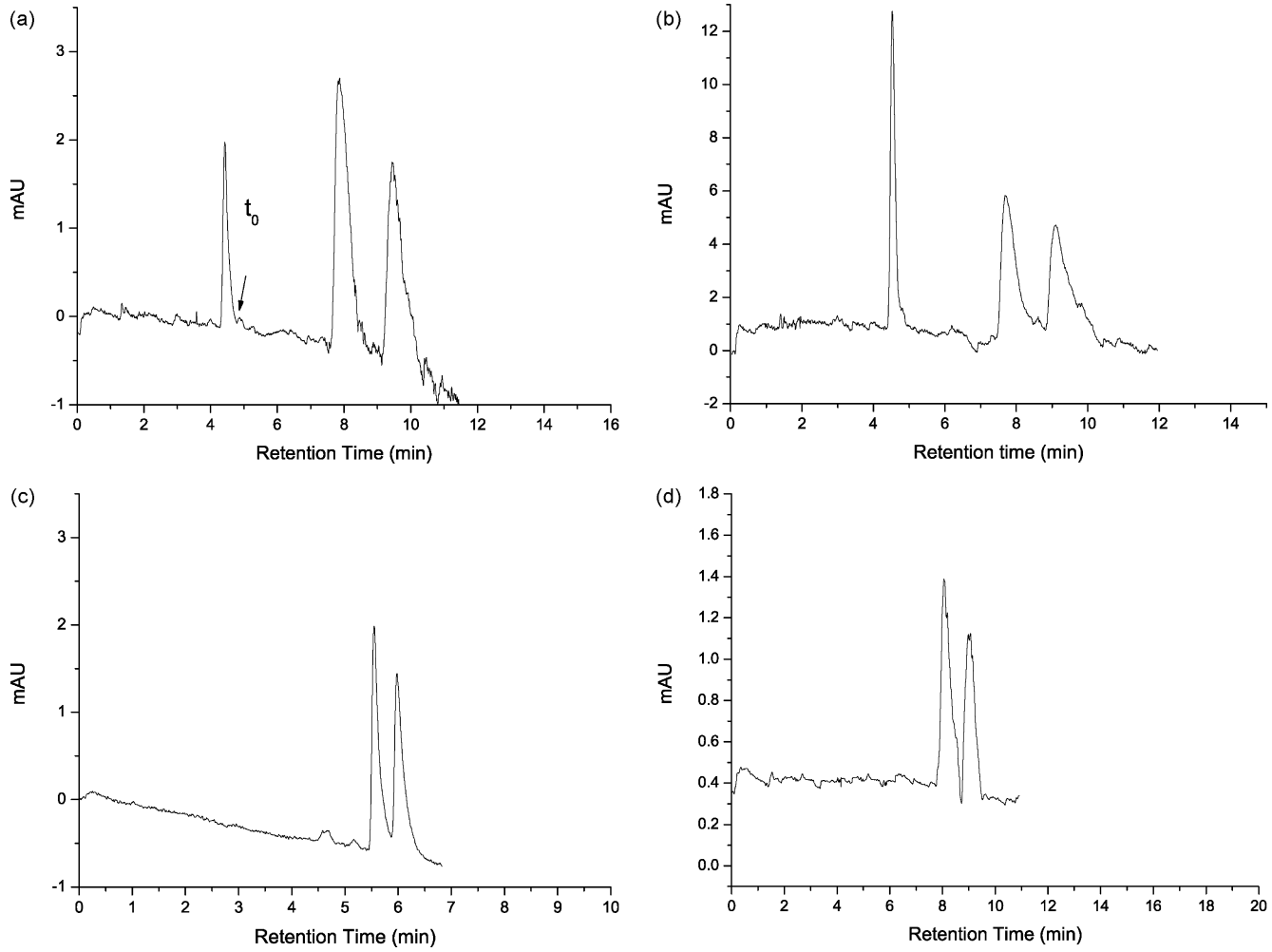

Fig. 3. Enantioseparation on CDMPC-bonded silica monolithic capillary column. Experimental conditions—solutes: (a) tetrahydropalmatine; (b) Tröger's base; (c) pindolol; (d) indapamide; other conditions were the same as in Table 1.

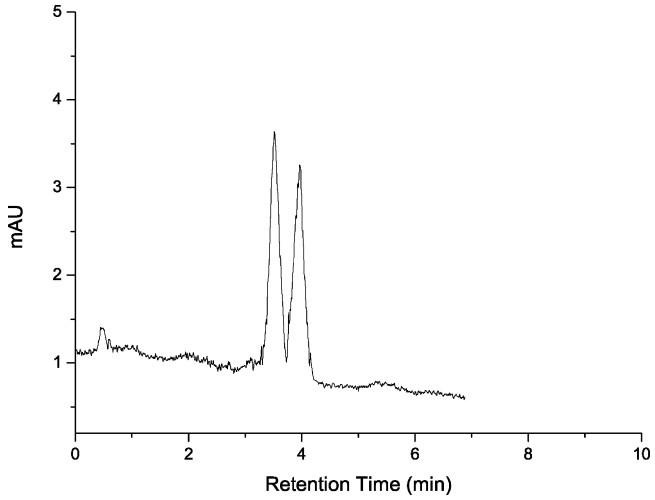

Fig. 4. Electrochromatogram for fast separation of indapamide. Experimental conditions: applied voltage, 25 kV; other conditions were the same as described in Table 1.

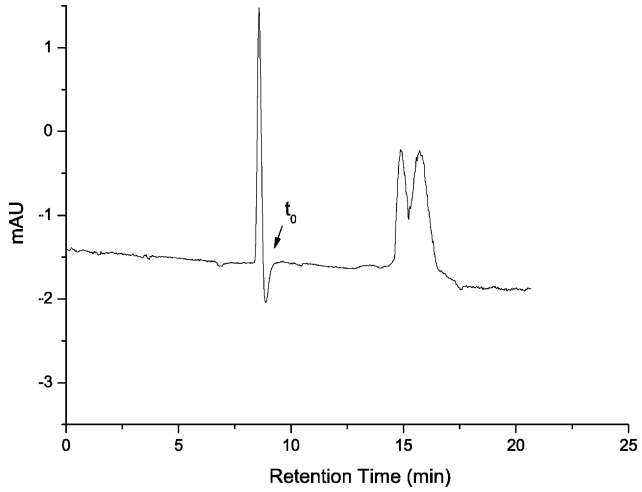

Fig. 5. The enantioseparation of praziquantel on a CDMPC-bonded silica monolithic capillary column with THF as MPM. Experimental conditions: mobile phase, 40% THF in 4 mM phosphate buffer (pH 6.8); other conditions were the same as in Table 1.

[36] has been achieved in CLC. The cellulose and amylose derivatives-bonded CSP silica monolithic column via the radical polymerization was reported by Chankvetadze et al. [37]. The separation efficiencies on our prepared CSP in CEC mode were not much higher than those in CLC by Chankvetadze et al. [37]. However, in our experiment, the obtained resolutions for Tröger's base and benzoin were both higher. Other racemates were also different from that in literature [37] and good recognition ability was obtained too. The fast separation on our bonded-type CSP in CEC could be conveniently realized without the loss of enantioselectivity by simply increasing the applied voltage. While, the fast separation in CLC will require the rise of the flow rate with the increase of the column pressure, which will consequently result in the problem of system stability. Additionally, the method for the immobilization of CSP in this work was different from that described by Chankvetadze et al. [37], where the polymerization of crosslinked polysaccharide derivatives and vinyl groups on the surface of the silica monolith was applied for immobilization. In this work, the approach for the covalent bonding of cellulose derivative on the silica monolithic matrix represents an efficient way for obtaining a chiral stationary phase with a good stability, and also it is the first trial to use this covalently bonded CSP in CEC for enantioseparation.

3.4. Enantioseparation in CEC with THF as mobile phase modifier (MPM)

The drawback of cellulose derivative-coated stationary phase for enantioseparation was the dissolving and swelling of cellulose derivative in some solvents, such as THF, chloroform, acetone, etc. [39]. Thus, the use of these solvents was strictly limited as mobile phase modifier. However, the restriction of using these solvents will also limit the further method development because they not only posses the good solubility for many analytes but also the ability in expanding or alternating the chiral recognition in some cases. The covalent bonding of cellulose derivative on silica monolithic matrix is expected to overcome this drawback, and the use of the above-mentioned solvents would provide better solubility of enantiomers and different separation selectivity [25].

In this work, the THF was used as the MPM for the enantioseparation of prazquantel on the CDMPC-bonded silica monolithic capillary column in CEC. The obtained result is illustrated in Fig. 5. As seen in figure, the prazquantel could be partially resolved (electrochromatographic data also listed in Table 1). This confirmed that the covalent bonding of cellulose derivative on a silica monolithic capillary column did offer a good stability for using the THF in chiral separation. In a comparison of using ACN and THF as MPM, the magnitude of R_s was improved from 0.58 for ACN to 0.98 for THF. From Fig. 5, it can also be told that the magnitude of EOF was notably lower with THF as MPM in mobile phase than that with ACN as MPM as shown in Fig. 3a. This could be explained that the viscosity of THF was higher than that of ACN, which correspondingly resulted in the decrease of EOF in CEC.

Although THF was effective for the enhancement of separation selectivities for some enantiomers, this could not be always successful to all cases. For example, benzoin could be well resolved when ACN was used as MPM, but could not be separated when THF was used.

In addition, no significant decline of resolution factors and efficiencies was observed after the columns being used under mobile phase containing THF as modifier. Also, no shrinking and swelling of the column beds were observed. This indicated good stability and mechanical strength of the prepared CSP which benefited from the nice skeleton offered by monolithic silica capillary columns and the advantage of the immobilized CDMPC. The broadened choice of solvents as MPM offered by the bonded CSP represents an advantage in method development. For the separation of enantiomers on the bonded cellulose derivative CSP, if the frequently used MPMs were not effective, some unusual solvents such as THF can be used as alternatives.

The repeatability and reproducibility of the prepared CSP were also examined in this work by using the Tröger's base as the test analyte and the thiourea as the marker of EOF with the mobile phase containing 40% ACN and 4 mM phosphate buffer (pH 6.8). The relative standard deviations (RSDs) for the EOF and the resolution of analyte were less than 1.8% and 4.8% ($n = 3$), respectively, which indicates the good repeatability of the prepared novel OT column in the separation of racemate. The column-to-column reproducibility was also investigated to evaluate the preparation variation of CSP columns. The RSDs of EOF and resolution were less than 4.6% ($n = 3$) and 6.9% ($n = 3$), respectively, for estimated with three prepared columns, which confirmed the good reproducibility in column preparation.

4. Conclusion

The cellulose derivative, CDMPC, could be successfully immobilized on a silica monolithic capillary column via the covalent bonding modification. The CDMPC-bonded silica monolithic capillary column showed favorable mass transfer kinetics and good enantioselectivity in separation of enantiomers by CEC, such as neutral and basic enantiomers. Six pairs of enantiomers were tested and five of them were successfully resolved. In addition, the CDMPC-bonded chiral stationary phase could be used to perform the enantioseparation of praziquantel even using the strong solvent-THF as the mobile phase modifier in CEC, and a better separation was observed when the MPM was changed from ACN to THF. The cellulose derivatives attached silica monolith also appears to be stable under mobile phases containing THF or ACN. These confirm the advantage of using the bonded CSPs over the coated ones.

Acknowledgments

The financial supports from the Natural Science Foundation of China (No. 20475054) and High-Tech Program Grant from CAS (KGCX2-SW-213-04) to Dr. H. Zou, and the Hundred Talent Program of CAS to Dr. R. Wu are gratefully acknowledged.

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