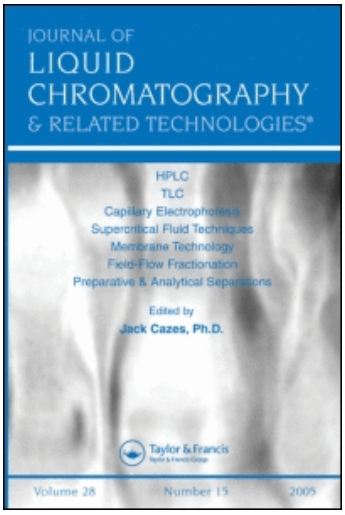

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

ENANTIOSEPARATION USING A CELLULOSE-BASED STATIONARY PHASE BY CAPILLARY LIQUID CHROMATOGRAPHY

Yi-Jun Zhang[a]; Yu-Ping Zhang[a]; Qi-Rong Duan[a]; Lian-Yang Bai[b]; Jun Chen[ab]; Xiao-Mao Zhou[b]
[a] Henan Institute of Science and Technology, Xinxiang, P. R. China [b] Pesticide Research Institute, Hunan Agricultural University, Changsha, P. R. China

Online publication date: 01 December 2010

To cite this Article Zhang, Yi-Jun , Zhang, Yu-Ping , Duan, Qi-Rong , Bai, Lian-Yang , Chen, Jun and Zhou, Xiao-Mao(2010) 'ENANTIOSEPARATION USING A CELLULOSE-BASED STATIONARY PHASE BY CAPILLARY LIQUID CHROMATOGRAPHY', Journal of Liquid Chromatography & Related Technologies, 33: 19, 1733 — 1744
To link to this Article: DOI: 10.1080/10826076.2010.526823
URL: http://dx.doi.org/10.1080/10826076.2010.526823

Journal of Liquid Chromatography & Related Technologies, 33:1733–1744, 2010

ISSN: 1082-6076 print/1520-572X online
DOI: 10.1080/10826076.2010.526823

ENANTIOSEPARATION USING A CELLULOSE-BASED STATIONARY PHASE BY CAPILLARY LIQUID CHROMATOGRAPHY

Yi-Jun Zhang,[1] Yu-Ping Zhang,[1] Qi-Rong Duan,[1] Lian-Yang Bai,[2] Jun Chen,[1,2] and Xiao-Mao Zhou[2]

[1]*Henan Institute of Science and Technology, Xinxiang, P. R. China*
[2]*Pesticide Research Institute, Hunan Agricultural University, Changsha, P. R. China*

□ *A 3,5-dimethylphenylcarbamate derivative of cellulose immobilized onto the aminopropyl-modified silica gel was used as a chiral stationary phase and separation of five enantiomers was successfully performed by a home-made chiral capillary liquid chromatography system. Different chromatographic parameters such as resolution, column efficiency, and retention time were comparatively investigated using three chiral stationary phases with different coatings. The key factors which may influence the performance of the chiral stationary phases were evaluated by varying the composition of the mobile phase and its flow rate. It was illustrated that the self-installed chiral separation system was characterized by simplicity, high resolution, ease for practical usage, and could be handily applied for enantioseparation at the conventional laboratory.*

Keywords cellulose tris(3,5-dimethylphenylcarbamate), capillary column, capillary liquid chromatography, chiral stationary phases, enantiomer separation, slurry packing

INTRODUCTION

Chiral separation has received considerable attention in many fields, particularly the pharmaceutical and pesticide industries.[1] Some analytical techniques have been developed for the enantioseparations such as high-performance liquid chromatography (HPLC), gas chromatography (GC), supercritical fluid chromatography (SFC), thin-layer chromatography (TLC), capillary electrophoresis (CE), capillary electrochromatography (CEC), and others.[2,3] For HPLC, the separation of enantiomers could be realized through either chiral mobile phase additives or chiral stationary phases. The chiral mobile phase approach represents a simple and flexible alternative, which is, however, not always applicable. Since

Address correspondence to Dr. Yu-Ping Zhang, Henan Institute of Science and Technology, Xinxiang 453003, China or Prof. Lian-Yang Bai, Pesticide Research Institute, Hunan Agricultural University, Changsha 410128, China. E-mail: yupzhang@hotmail.com; blyang2006@163.com

the mobile phase containing the chiral selector cannot be reused, this technique cannot be applied with expensive reagents. The direct approach using columns with chiral stationary phases is more convenient and also applicable for separations but requires a large collection of expensive stationary phases and hazardous organic solvents. Downscaling of 4.0–4.6 mm ID HPLC columns to capillary format seems to be a promising method for this approach, but the difficulties involving capillary packing and frit preparation need to be overcome in the application of capillary liquid chromatography (CLC).

In this paper, a simple chiral separation system was constructed in our lab. The chiral stationary phase coated with different concentration of the cellulose tris(3,5-dimethylphenylcarbamate) (CDMPC) was packed in the capillaries, respectively, using the sintered steel powders as the inlet frit and the packed stationary phase itself as the outlet frit. Five enantiomers, namely 2-methyl-9-anthratenemethanol, trans-stilbene oxide, 1-indanol, 2,2,2-trifluoro-1-(9-anthryl) ethanol and benzyl mandelate metazoline were tested in order to verify the recognition capability of the polysaccharide derivative. The effect of experimental parameters such as mobile phase composition, flow rate, and loading of the coated chiral selector on retention, resolution, and peak efficiency was investigated, respectively.

EXPERIMENTAL

Instrumentation

CLC experiments were carried out by using laboratory assembled instrumentation. It included a HP1100 Series HPLC pump system (Agilent Technologies, Inc., Walbronn, Germany), equipped with an injector with a 20 μL quantitative tube (Rheodyne 7725i), and an on-capillary column detector with a changeable ultraviolet-visible wavelength in the range of 190–700 nm (Beijing Cailu Scientific Instrument Ltd., China). A T union was used for separation delivering appropriate amounts of the mobile phase. The prepared separation column inlet was installed at one outlet of the T union using a PEEK sleeve (0.5 mm i.d., 1.6 mm o.d.) and a screwed joint. Another capillary (3 m × 100 μm i.d) for splitting the injection sample and mobile phase was connected to the other outlet of the T union. Chromatograms were recorded using the computer software N2000 chromatography data system supplied by Zhida Information Engineering Ltd., Zhejiang University, China. A pneumatic pump (RPL-ZD10, Dalian Replete Scientific Instrument Co., Ltd, Dalian, China) and an ultrasonic bath (KQ-500E, Kunshan Ultrasonic Instrument Co., Ltd, Kunshan, China) were used to drive solvent and slurry into the capillary during

column preparation. An FEI QUANTA 200 scanning electron microscope (Philips-FEI Corporation, Netherlands) was used to study the morphology of monolith. A capillary with the monolith was sectioned into 10 mm segments without sputtering with gold prior to SEM analysis. An analytical balance (Beijing Sartorius Instrumental Limited Company, max = 120 g, d = 0.1 mg) was used to determine the splitting ratio.

Materials and Chemicals

Fused-silica capillaries (100 μm i.d., 375 μm o.d.) were purchased from Yongnian Ruipu Optic Fiber Plant (Yongnian, Hebei Province, China). Stainless steel powders (under 500-mesh) were purchased from Beijing Gelubo Alloy Material Limited Company. Spherical silica (Akzo Nobel, Sweden, particle size 5-μm, average pore size 10 nm), 3-aminopropyltriethoxysilane (Aldrich, USA), toluene (Junsei, Japan), microcrystalline cellulose (Aldrich, USA), 3,5-dimethylphenyl isocyanate (TCI, Japan), and pyridine (Junsei, Japan). Chiral compounds including α-methyl-9-anthratenemethanol, 2,2,2-trifluoro-1-(9-anthryl)enthanol, 1-Indanol, trans-Stilbene oxide, and benzyl mandelate were purchased from sigma company. Acetonitrile (ACN), methanol, isopropanol (IPA), and hexane (HEX) were purchased from Beijing Bailingwei Chemical Reagent Company and Tianjing Chemical Reagent Company, China.

Preparation of Stationary Phase

Preparation of Aminopropyl-Modified Silica Gel

The aminopropyl-modified silica gel was prepared as described previously.[4–8] 6g of 5-μm silica gel and 50-ml toluene were added to a 100-ml round bottom flask. From the resulting slurry, the water was removed azeotropically by using a Dean–Stark water trap. After the complete removal of water, 12 ml of 3-aminopropyltriethoxysilane was added to the slurry, and the whole mixture was heated to reflux for 3 days with magnetic stirring. The modified silica gel was isolated by filtration and washed with toluene, methanol, diethyl ether, petroleum ether, and dried under vacuum condition at 50°C for 24 h.

Synthesis of Cellulose Tris(3,5-dimethylphenylcarbamate)

Cellulose tris(3,5-dimethylphenylcarbamate) was synthesized as previously reported.[9–12] 1 g of dried cellulose was refluxed in 80 ml of dry pyridine for 24 h. 10 ml of 3,5-dimethylphenyl isocyanate was added dropwise to the cellulose suspension under magnetic stirring and the reaction was continued for 48 h under reflux. The final product was isolated as the methanol-insoluble fraction and purified by reprecipitation from an

acetone solution. The solid was filtered, washed several times with methanol, and finally dried in a vacuum oven at 60°C for 24 hours. The yield was 80% in our experiments.

Chiral Selector Coated onto Modified Silica Particles

Firstly, cellulose tris(3-chloro-4-methylphenylcarbamate) (0.020 g) was dissolved in 30 mL tetrahydrofurane (THF) by ultrasonic bath; placed the aminopropyl-modified silica gel (0.400 g) in a round-bottom flask; added the solution (10 mL) to silica gel by drops; shook the flask to uniformly coat the polysaccharide derivative (5%, w/w) on the silica surface; then, dried the silica gel (CSP_1) under vaccum at 60°C for 8 h. The following CSPs were repeatedly prepared using aminopropyl-modified silica gel coated with 10% and 15% (w/w) polysaccharide derivative (CSP_2, CSP_3), respectively.[4–14] Figure 1 shows a schematic representation of CDMPC on silica gel.

High Pressure Slurry Packing of CSP

The capillary chiral columns were prepared by using the slurry packing method as described previously.[15] The procedure for the outlet frit preparation was developed by our laboratory, which was described as follows: A small number of stainless steel powders were placed into a 1.0 mL vial. One end of each polyimide-coated fused-silica capillary was tapped into the vial to allow some small steel particles to be forced into the head of each capillary. The frit of sintered powders could be formed at the capillary head by burning butane, which was produced by a commercial welding torch. Most metal powders became agglomeration in the capillaries after being burned for about 10–30 s. Some small holes or flaws could be observed when the fused metal powders were refrigerated to room temperature. Due to the rapid thermal swelling, stainless powder beads would fuse to form a "steel arch" structure and the swelling powders could be wedged into the capillary wall. The sintered steel powders and the "stone arch" between the steel mesh and capillary wall could hold back the stationary phase. The successful frit did not result in any discernable deformity or shrinkage in the fused-silica capillaries under a light microscope. A fine spray under

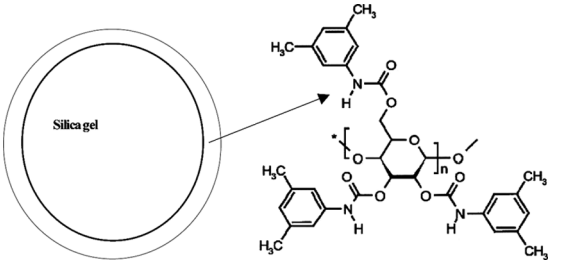

FIGURE 1 Schematic representation of CDMPC on the silica surface.

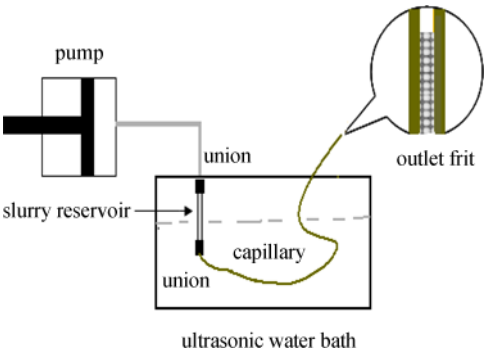

FIGURE 2 Schematic diagram to pack the chiral separation column. (Figure available in color online.)

pressure should be observed for successful frits indicating minimum flow resistance and good porosity to allow maximum packing material flow. If no such spray was observed the frit was removed by cutting, and the procedure was repeated so as to obtain a frit with a desired length and porosity. Then, the capillary with a prepared frit was slurry packed with 5 μm CSP silica gel in a 90 mm × 1 mm i. d. stainless steel column as slurry reservoir. The other capillary end was connected to the slurry reservoir by means of a screwed joint and a piece of 1/16" PEEK tubing as sleeve. The slurries (0.0150 g CSP in 100 μL carbon tetrachloride/1–4-dioxane, 1/2, v/v) were sonicated for 5 min and filled bubble-free into the slurry reservoir. After rapidly closing the reservoir, the capillary was immersed into an ultrasonic bath while the capillary with the end fitting was kept outside in a vertical position. The packing procedures could be carried out by the flush of slurry liquid under the pressure of 40 MPa, followed by propanol within 1 h (see Figure 2). After packing, another frit was sintered at the suitable position by heating the packing CSP in the flame of welding torch. It should be noted that the pressure was slowly reduced to atmospheric pressure during several hours, and then the filled capillary was dismounted. The packing procedure could be viewed visually by means of a stereo microscope. The excess of stationary phase was removed by flushing the capillary with the mobile phase also providing a fast equilibration of the material. A window was created by burning the polyimide coating of the capillary at the desired capillary position.

RESULTS AND DISCUSSION

Home-Made Chiral Separation System

The chromatographic performance of the packed CSPs was compared with the results of our self-installed capillary chiral liquid chromatography

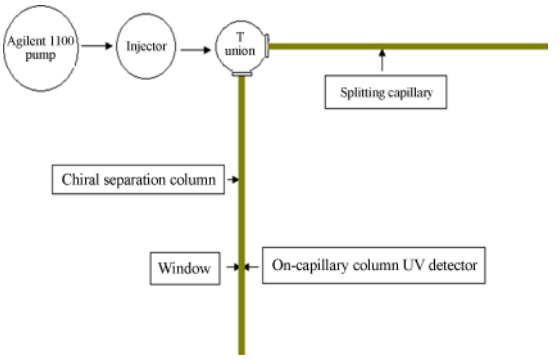

FIGURE 3 Schematic diagram of capillary chiral separation system. (Figure available in color online.)

system.[15] In general, sample injection was carried out by a nano-injector in a commercial capillary-HPLC instrument. In our experiment, eluent flow through the capillary column was controlled by a custom built adjustable flow splitter based upon a T-piece connector with a capillary (see Figure 3). The backpressure enforced on the separation column could be adjusted by the change of another capillary inner diameter and length. The splitting capillary was linked to the waste container under the atmosphere. Splitting ratio was calculated by weighing the eluate from the capillary outlets, which was collected in a sealed vial. Generally, the volume eluated from the splitting capillary was greater than that eluated from the separation column due to the presence of larger back pressure from the latter. The usage of on-column detector could effectively improve the sensitivity and avoid the peak broadening due to the dead volume in the conventional flow cell of UV detector.

Effect of Coating Amount of CDMPC on the Chromatographic Performance

The choice of the coating thickness has a great influence on column performances such as the retention time, resolution, and column efficiency. The CDMPC coated the silica gel at high loads may also be associated with an ordered structure. Such an associated CDMPC may have a conformation or orientation different from that of the CDMPC coated at lower amounts.[16] This may be the reason why chiral recognition can be affected by the coating amount of CDMPC on silica gel. If the coating is too thin, no enantiomer resolution can be achieved, possibly because of stationary phase overloading. On the other hand, with thicker coatings, several problems are encountered: The high viscosity of the coating solution makes the coating process difficult and slow. By changing the concentration of CDMPC for

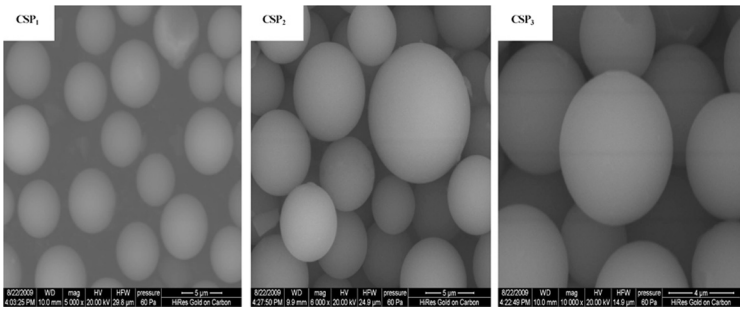

FIGURE 4 SEM of the prepared chiral stationary phases.

coating on the aminopropyl-modified silica gel, the thickness of CDMPC film as the CSPs can be controlled correspondingly in our experiments. Figure 4 shows a schematic representation of CDMPC on silica gel with different coating amount; no obvious conglobation was found among the silica particles (CSP1, CSP2, and CSP3) after being coated. Moreover, the better and even coatings are obtained for the silica gel coated with 5% (*w*/*v*) of CDMPC in THF. Figure 5 represents the obtained enantioseparation effects of five compounds using the silica gel coated with 5, 10, and 15% (*w*/*v*) CDMPC as the CSPs, respectively. It can be seen that the resolution and the column efficiency of the enantiomers are obviously different for different CSPs, and baseline separation of 2,2,2-trifluoro-1-(9-anthryl) ethanol and 1-Indanol could be achieved using three CSPs, respectively. However, the decrease of the column efficiency was obviously obtained when the coating concentration of CDMPC equal to 10%. Compared with columns 1 and

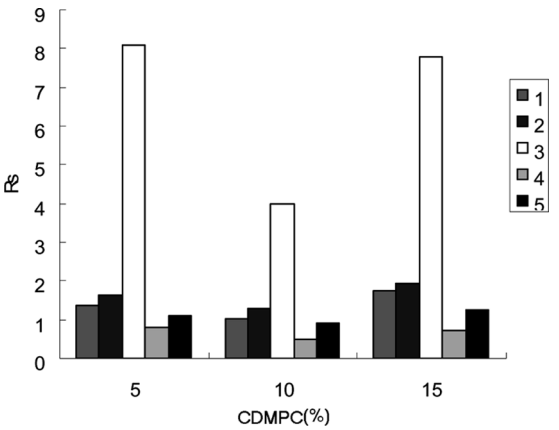

FIGURE 5 Effect of CDMPC loads on the resolution for five enantiomers. Numbers 1–5 stand for α-methyl-9-anthratenemethanol, 2,2,2-trifluoro-1-(9-anthryl)enthanol, 1-Indanol, trans-Stilbene oxide, benzyl mandelate, respectively.

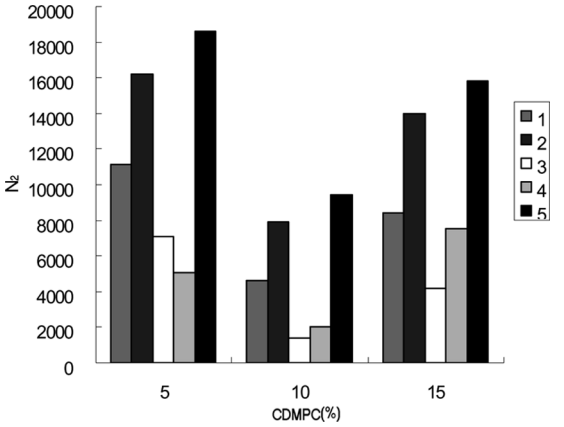

FIGURE 6 Effect of CDMPC coating amount on theoretical plates of last peak to five enantiomers.

3, the column efficiency for the last peak of five chiral compounds, respectively, sacrificed for the column 2 (see Figure 6). It should be noted that the higher the concentration of CDMPC used for coating, the more difficult and more time-consuming for the enantioseparation. Table 1 shows that the longest retention times for five enantiomers were generally obtained using the CSP3, respectively. Thus, the final concentration of 5% w/v CDMPC in THF was employed for coating the silica gel for the subsequent experiments.

Effect of Flow Rate on the Chromatographic Performance

When the flow rate displayed in the collected software was changed from 0.2000 to 0.7000 mL/min with a stepwise rate of 0.1 mL/min, the actual flow rate in the capillary tube increased from 0.159 to 0.541 μL/min with a kept mobile phase percentage (HEX:IPA = 90:10) and back

TABLE 1 Retention Time for Five Enantiomers on the CSP_1, CSP_2 and CSP_3

		CSP_1 (min)		CSP_2 (min)		CSP_3 (min)	
Enantiomer	Mobile phase	t_1	t_2	t_1	t_2	t_1	t_2
1	a	5.378	6.161	5.147	6.132	7.274	8.826
2	a	8.126	9.557	7.350	9.04	11.508	14.469
3	b	2.259	2.821	2.342	2.879	2.502	3.211
4	c	2.473	2.703	2.592	2.808	2.909	3.163
5	c	9.415	10.113	8.687	9.525	12.002	13.220

Mobile phases: (a) HEX/IPA 90:10(v/v), (b) HEX/IPA 70/30(v/v), (c) HEX/IPA 95/5(v/v); Flow rate: 0.5 mL/min, room temperature.

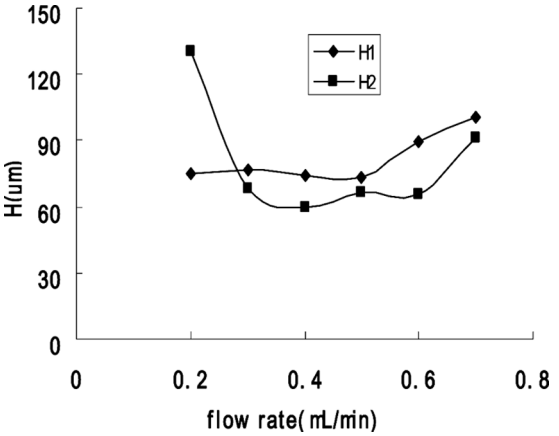

FIGURE 7 Van Deemter equation plots for 2,2,2-trifluoro-1-(9-anthryl) enthanol.

pressure of the prepared column increased from 1.2 to 4.6 MPa, with a splitting ratio changed in the range of 1213 to 1369. In order to investigate the effects of flow rate on the theoretical plate height (*H*) 2,2,2-trifluoro-1-(9-anthryl) ethanol was selected for the investigation. Figure 7 described the relationship between the set flow rate in the capillary tube and the *H* values. The curves were plotted using 6 points, with each point repeated three times. The relevant *H* values changed in the range of 73 to 110 μm for the first eluated peak and 59 to 131 μm for the second peak. This suggested that the H values were increased when the flow rates were greater than 0.4 mL/min, and it was in accordance with van Deemter equation, the more the resistance to mass transfer at higher flow rates, the less the column efficiency.

Effect of Mobile Phases on the Chromatographic Performance

Figure 8 showed that the *H* values were changed with the percentage of mobile phase (HEX/IPA *v*/*v*) with a constant flow rate of 0.5 mL/min. The curves had similar trends over the full range of the percentage of mobile phase. The curves were also plotted using 6 points, with each point repeated at least three times, respectively. With the change of mobile phase percentage in the range of 92:8 to 82:18 with a stepwise change of 2% IPA, the decreased trends of *H* values could be observed with the lowest *H* equal to 57 μm throughout all experiments, while the retention time for each peak were obviously decreased from 11.88 min to 5.93 min with the increase of polar component (IPA) from 8% to 18%. Furthermore, the dead time by the prepared column almost kept unchanged throughout all experiments, which was obtained by taking 1,3,5-Tri-tert-butylbenzene, an unretained

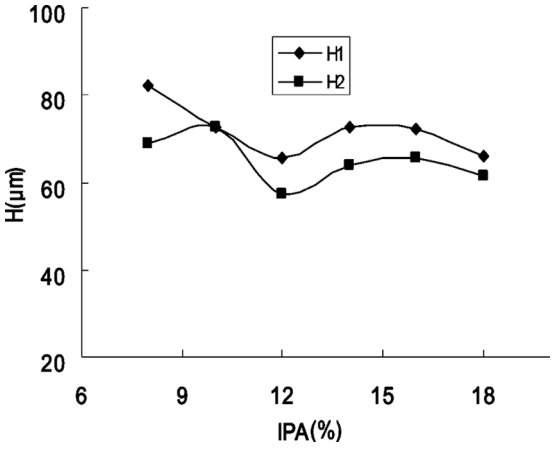

FIGURE 8 Effect of isopropanol percentage on *H* for 2,2,2-trifluoro-1-(9-anthryl) enthanol.

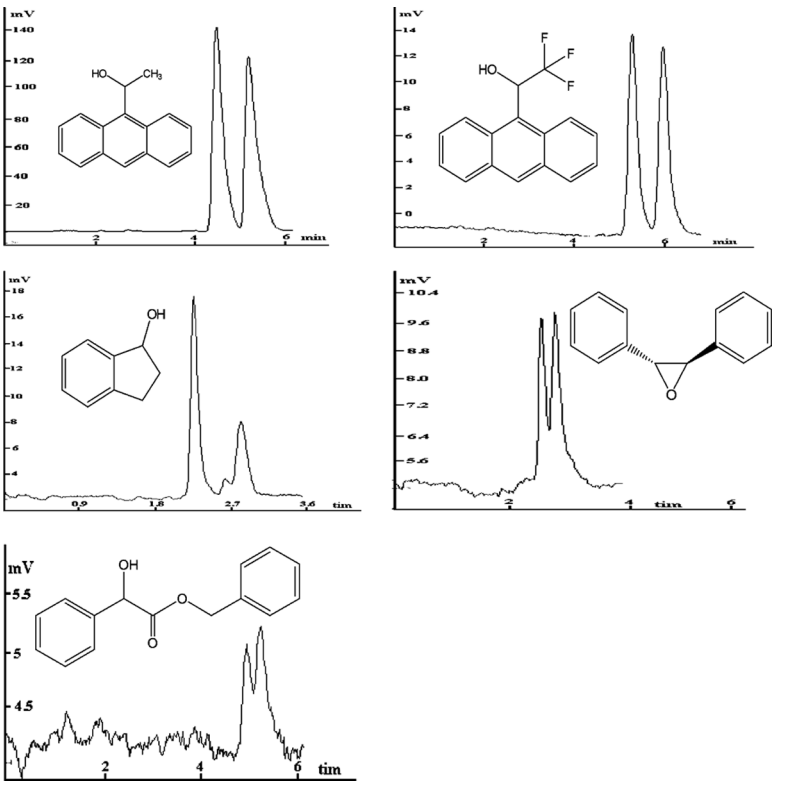

FIGURE 9 Chromatograms of five chiral compounds using the optimal conditions. Experimental conditions: room temperature, flow rate = 0.5 mL/min, the mobile phases for enantiomers 1–5 are HEX: IPA = 90:10, 82:18, 70:30, 95:5, and 85:15, respectively.

component as the marker. The relative standard deviation of retention time for the marker was calculated to be 1.21% ($n = 6$), which indicated no obvious change of dead volume and no bleeding of the CSP particles throughout all experiments. Moreover, some typical chromatograms using the optimal conditions for five enantiomers were shown in Figure 9. Enantiomers 1, 2, and 3 possessed the better separation efficiency than enantiomers 4 and 5, and the different chiral discrimination was probably attributed to the structure difference for the separated enantiomers.

CONCLUSIONS

CDMPC was coated on the porous silica gels and used as the CSPs for capillary high-performance liquid chromatographic separation of enantiomers. The amount of CDMPC adsorbed on the silica gel influenced the chiral recognition ability; however, this was dependent on the racemate that was examined. No apparent peak dispersion and bubbles were observed over long chromatographic operations using our self-prepared chiral columns, and the actual flow rate in the capillary tube could be adjusted by the simple splitting system. It was an effective, simple, and economical method for capillary separation using the developed analytical HPLC instead of commercial nano-HPLC.

ACKNOWLEDGMENT

The financial support by the Scientific Research Foundation for the Returned Overseas Chinese Scholars, State Education Ministry, and the National Natural Science Foundation of China (Nos. 30070506, 30771436) are gratefully acknowledged.

REFERENCES

1. Gübitz, G.; Schmid, M. G. Recent Advances in Chiral Separation Principles in Capillary Electrophoresis and Capillary Electrochromatography. *Electrophoresis* **2004**, *25*, 3981–3996.
2. Okamoto, Y.; Kaida, Y. Polysaccharide-based Chiral Stationary Phases for High-Performance Liquid Chromatographic Enantioseparation. *J. Chromatogr. A* **2001**, *906*, 105–125.
3. Gerald, G.; Martin, G. S. Chiral Separation by Chromatographic and Electromigration Techniques. *Biopharm. Drug Dispos.* **2001**, *22*, 291–336.
4. Okamoto, Y.; Kawashima, M.; Yamamoto, K.; Hatada, K. Useful Chiral Packing Materials for High-Performance Liquid Chromatographic Resolution Cellulose Tribenzoate and Tribenzoate Coated on Macroporous Silica Gel. *Chem. Lett.* **1984**, *13*, 739–742.
5. Okalmoto, Y.; Yashima, E. Polysaccharide Derivatives for Chromatographic Separation of Enantiomers. *Angew. Chem. Int. Ed.*, **1998**, *37*, 1020–1043.
6. Chankvetadze, B.; Yamamoto, C.; Okamoto, Y. Enantioseparation of Selected Chiral Sulfoxides Using Polysaccharide-Type Chiral Stationary Phases and Polar Organic, Polar Aqueous–Organic and Normal-Phase Eluents. *J. Chromatogra. A* **2001**, *922*, 127–137.

7. Fanali, S.; Orazioa, G. D.; Lomsadzeb, K.; Chankvetadzeb, B. Enantioseparations with Cellulose Tris(3-chloro-4-methylphenylcarbamate) in Nano-Liquid Chromatography and Capillary Electrochromatography. *J. Chromatogr. B.* **2008**, *875*, 296–303.
8. Persson, B.; Anderson, S. Unusual Effects of Separation Conditions on Chiral Separations. *J. Chromatogr. A 2001*, *906*, 195–203.
9. Francottee, R.; Zhang, T. Supramolecular Effects in the Chiral Discrimination of Metamet Hylbenzoyl Cellulose in High-Performance Liquid Chromatography. *J. Chromatogr. A* **1995**, *218*, 257–266.
10. Okamoto, Y.; Kaida, Y. Resolution by High-Performance Liquid Chromatography Using Polysaccharide Carbamates and Benzoates as Chiral Stationary Phase [J]. *J Chromatogr A* **1994**,*666*, 4032409.
11. Okamoto, Y.; Kawashima, M.; Hatada, K. Chromatographic Recognition Controlled Chiral Recognition of Cellulose Triphenylcarbamate Derivatives Supported on Silica gel. *J. Chromatogr.* **1986**, *363*, 173–186.
12. Kubota, T.; Yamamoto, C.; Okamoto, Y. Tris(cyclohexylcarbamate) of Cellulose and Amylose as Potential Chiral Stationaryphases for High-Performance Liquid Chromatography and Thin-Layer Chromatography. *J. Am. Chem. Soc.* **2000**, *122*, 4056–4059.
13. Chankvetadze, B.; Ikai, T.; Yamamotob, C.; Okamoto, Yoshio. High-Performance Liquid Chromatographic Enantioseparations on Monolithic Silica Columns Containing a Covalently Attached 3,5-dimethylphenylcarbamate Derivative of Cellulose. *J. Chromatogra. A.* **2004**, *1042*, 55–60.
14. Krause, K.; Girod, M.; Chankvetadze1, B.; Blaschke, G. Enantioseparations in Normal- and Reversed-Phase Nano-High Performance Liquid Chromatography and Capillary Electrochromatography Using Polyacrylamide and Polysaccharide Derivatives as Chiral Stationary Phases. *J. Chromatogr. A* **1999**, *837*, 51–63.
15. Gong, W. J.; Zhang, Y. P.; Chen, N.; Wang, A. R.; Tang, B.; Shi, M. W.; Qu, L. B. Fritless Column for Capillary HPLC Prepared by Immobilizing Octadecylsilane Particles in an Organic Polymer Matrix. *J. Liq. Chromatogr. & Rel. Technol.* **2009**, *32*, 1847–1861.
16. Yashima, E.; Sahavattanapong, P.; Okamoto, Y. HPLC Enantioseparation on Cellulose Tris(3,5-dimethylphenylcarbamate) as a Chiral Stationary Phase: Influences of Pore Size of Silica Gel, Coating Amount, Coating Solvent, and Column Temperature on Chiral Discrimination. *Chirality* **1996**, *8*, 446–451.