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# Synthesis of chiral stationary phases with radical polymerization reaction of cellulose phenylcarbamate derivatives and vinylized silica gel

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**Abstract**

Cellulose phenylcarbamate derivatives having methacrylate groups were synthesized with regioselective and non-regioselective procedures. These derivatives were chemically immobilized onto a vinylized silica gel, respectively, via a radical co-polymerization reaction. The immobilization was efficiently attained using a small amount of AIBN. The chiral recognition abilities of the prepared chiral stationary phases (CSPs) were evaluated by HPLC resolution of test enantiomers. It was observed that most of the enantiomers were completely resolved with markedly high column efficiency of 30,000–40,000 plates per metre for the eluted peaks. The effect of the amount of methacrylolyl chloride used for preparation on resolution was investigated. A direct comparison of the chiral recognition ability was made on the regioselectively and non-regioselectively prepared CSPs. In addition, the chemically bonded-type of CSPs were found to be relatively stable with addition of solvents such as tetrahydrofuran (THF) and chloroform into the mobile phase, which can lead to the dissolution of cellulose derivatives on the coated CSPs. Thus the choice of solvents used as the mobile phase is greatly extended and better resolution of several test enantiomers was observed on the prepared CSPs with THF and chloroform as a composition in the mobile phase. The batch-to-batch and run-to-run reproducibility was also discussed on the newly prepared CSPs.


*Keywords:* Chiral stationary phases, LC; Radical polymerization; Methacryloyl chloride; γ-(Trimethoxysilyl)propyl methacrylate

## 1. Introduction

Separations of enantiomers by high-performance liquid chromatography (HPLC) play a most important role now [1]. Polysaccharide derivatives coating onto silica matrices represents nowadays one of the most popular chiral stationary phases (CSPs) and these kinds of phases are commercially available [2–6]. However, the solubility of polysaccharide derivatives in a number of solvents limits the application range on the commercial chiral columns [7]. This problem can be solved by chemically fixing polysaccharide derivatives onto silica matrices. Preparation of chemically bonded cellulose and amylose phenylcarbamate-based CSPs have been reported by Okamoto and co-workers [8–10], Minguillón and co-workers [11–13] and other researchers [7]. However, these CSPs are not commercially available until now. Therefore, it is important to develop novel procedures for effective synthesis of the bonded-type of CSPs with polysaccharides. Recently, we have reported the synthesis of immobilized cellulose derivative-based CSPs with a bifunctional reagent of 3-(triethoxysilyl)propyl isocyanate, which was proved to be useful for rapid preparation [14].

In this study, a radical co-polymerization reaction between methacrylate groups is adopted for chemically bonding cellulose derivatives onto silica matrix. Series of CSPs with cellulose phenylcabamate and cellulose 3,5-dimethylphenylcarbamate were, respectively, prepared with non-regioselective and regioselective procedures, and their chiral resolving abilities were tested by HPLC separation of racemates under mobile phases of hexane/alcohol and hexane/tetrahydrofuran (THF) or chloroform/alcohol.

## 2. Experimental

### 2.1. Chemicals

Microcrystalline cellulose with a molecular mass-scale of 500–3800 was obtained from Serva (Heidelberg, Germany). Silica gel (Chromasil, 5 μm, 200 Å, 200 $m^2$/g)

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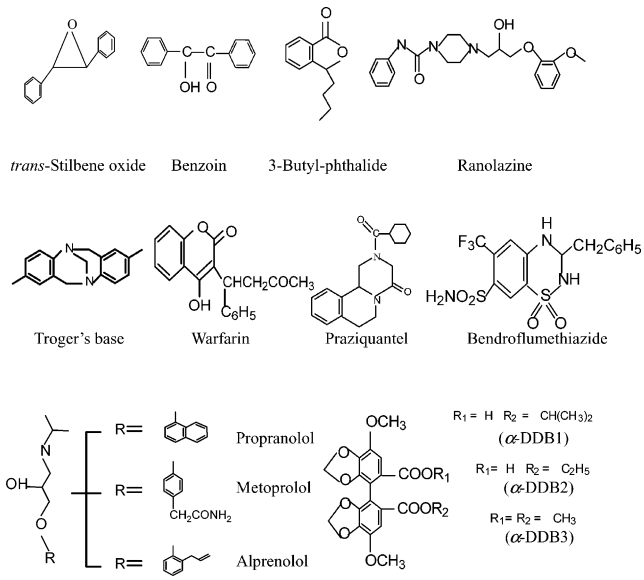


Fig. 1. Molecular structures of the test enantiomers.

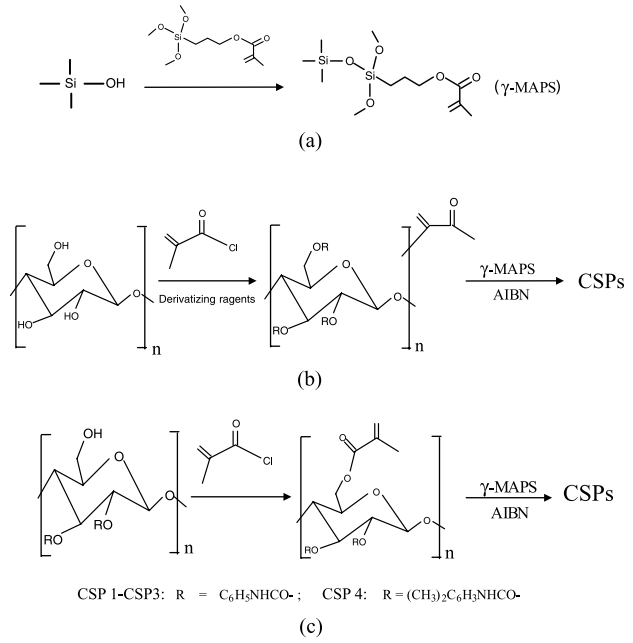


Fig. 2. Procedures for preparation of the bond-type of CSPs: (a) synthesis of γ-MAPS; (b) non-regioselective procedures; and (c) regioselective procedures.

was purchased from Akzo Noble AB (Nacka, Sweden). Methacryloyl chloride (MC) and γ-(trimethoxysilyl)propyl methacrylate were obtained from Acros (New Jersey, USA). AIBN (α,α′-azobisisobutyronitrile) was obtained from Sanpu Chemical Factory (Shanghai, China).

The racemic compounds of *trans*-stilbene oxide, benzoin, warfarin, praziquantel, alprenolol, metoprolol, propranolol, bendroflumethiazide, and Troger's base were all purchased from Sigma (St. Louis, MO, USA). Racemates of drug candidates A and B and ranolazine were obtained from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). α-Dimethyl dicarboxyl biphenyl derivatives of α-DDB1, α-DDB2 and α-DDB3 were obtained from Lanzhou Institute of Chemical Physics, Chinese Academy of Sciences. 3-Butyl-phthalide was obtained from CSPC Pharmaceutical Technology Co. Ltd. (Shijiazhuang, China). The molecular structures of these solutes are presented in Fig. 1.

### 2.2. Synthesis of vinylized silica gel (γ-MAPS)

As shown in Fig. 2(a), silica gel (6.0 g) was deposited into a 100 ml flask containing 40 ml dried toluene, then γ-(trimethoxysilyl)propyl methacrylate (8.0 ml) was added into the solution. The mixture was allowed to react at about 80 °C for 12 h. After that, the product was completely washed by toluene, ethanol, and methanol, respectively. Thus γ-MAPS was obtained. The results of elemental analyses are described in Table 2.

### 2.3. Preparation of CSPs with non-regioselective procedures

A schematic description of the procedures for preparing cellulose derivatives and CSPs bonded in non-regioselective procedures is shown in Fig. 2(b). Different amounts of methacrylolyl chloride (CSP1: 0.51 mmol; CSP2: 1.02 mmol) were, respectively, dissolved into flask containing 30 ml of pyridine, then 0.8 g of dried microcrystalline cellulose and 3.0 ml of phenyl isocyanate were added. The mixture was then allowed to react at 80 °C for 12 h under the protection of nitrogen. After that, the cooled solution was poured into a large amount of methanol, the insoluble fraction was isolated by centrifugation and washed carefully with methanol and dried under vacuum. Thus cellulose phenylcarbamate derivatives (I and II) with methacrylate groups at 2, 3 or 6 positions were prepared as listed in Table 1.

The synthesized cellulose phenylcarbamate derivative (0.5 g) was, respectively, dissolved into 15 ml THF with addition of γ-MAPS (2.0 g). After stirring for about 20 min, the solvent was evaporated under vacuum, thus cellulose derivatives were coated onto silica matrix. Then the solid mixture was dispersed into a 50 ml flask containing a 50 mg of AIBN. The mixture was stirred and reacted at about 100 °C for 2 h. After that, 30 ml pyridine was poured into the flask to completely dissolve the residues. The product was then collected by centrifugation and washed with THF and methanol. Thus CSP1 and CSP2 were prepared with amounts of methacrylolyl chloride of 0.51 and 1.02 mmol, respectively.

### 2.4. Preparation of CSPs with regioselective procedures

In this method, the methacrylate groups were introduced onto the 6-position of the glucose units through

Table 1
Preparation and characterization of cellulose phenylcarbamate derivatives

| Cellulose derivative | Procedure | Derivatizing reagent | Amount of MC used (mmol) | Elemental analyses (%) | |
|---|---|---|---|---|---|
| | | | | C | N |
| I | Non-reg[a] | PI[b] | 0.51 | 59.9 | 7.20 |
| II | Non-reg | PI | 1.02 | 61.2 | 7.50 |
| III | Reg[c] | PI | 1.02 | 62.8 | 7.38 |
| IV | Reg | DMPI[d] | 1.02 | 64.8 | 7.32 |

[a] Non-regioselevctive.
[b] Phenyl isocyanate.
[c] Regioselective.
[d] 3,5-Dimethylphenyl isocyanate.

the regioselective reaction of methacrylolyl chloride and primary hydroxyl groups of cellulose. For this aim, cellulose 2,3-bis(phenylcarbamate) and cellulose 2,3-bis(3,5-dimethylphenylcarbamate) were prepared in advance, and the detailed procedures of preparation were previously reported in the literatures [9,15].

As shown in Fig. 2(c), 0.5 g of cellulose 2,3-bis(phenylcarbamate) (for CSP3) and cellulose 2,3-bis(3,5-dimethylphenylcarbamate) (for CSP4) were, respectively, dissolved in 30 ml pyridine, and methacryloyl chloride (1.02 mmol) was slowly added into the solution. The mixture was then allowed to react at about 70–80 °C for 12 h. After that, the solution was poured into a 500 ml beaker containing 300 ml methanol. Then, the insoluble fraction was collected by centrifugation and completely washed by methanol. Thus, cellulose 2,3-bis(phenylcarbamate)-6-methacrylate (III) and cellulose 2,3-bis(3,5-dimethylphenylcarbamate)-6-methacrylate (IV) were prepared, respectively. After that, cellulose 2,3-bis(phenylcarbamate)-6-methacrylate was dissolved into THF (15 ml), and then coated onto γ-MAPS as described in Section 2.3. The following procedures were the same as those described in non-regioselective procedures, thus the regioselectively prepared CSP3 was obtained. Repeating the above procedures, CSP4 was also prepared with the immobilization of cellulose 2,3-bis(3,5-dimethylphenylcarbamate)-6-methacrylate and γ-MAPS. The CSPs prepared in this study are listed in Table 2.

### 2.5. Apparatus and chromatography

The HPLC experiments were performed with a Waters 510 pump (Waters, Milford, USA), a Spectra-200 UV detector (Spectra-Physics, San Jose, CA, USA) and a WDL-95 workstation (National Chromatographic R&A Center, Dalian, China).

The CSPs were dispersed into a pure methanol and packed into stainless-steel columns (150 mm × 4.6 mm i.d.), respectively, by a slurry packing technique. Throughout this study, chromatographic separations were performed at ambient temperature with a flow rate of 0.5 ml/min. The mobile phases were filtered and sonicated prior to use. All the test solutes were detected at 254 nm except for warfarin at 280 nm.

## 3. Results and discussion

### 3.1. Preparation of the CSPs

The chemical fixations of cellulose derivatives onto silica matrices used as CSPs by means of radical polymerization reactions have been reported previously [7]. One of the approaches involved the co-polymerization of a cellulose tris(4-vinylbenzoate) with a γ-aminopropylsilica gel modified by acryloyl chloride [16]. Minguillón et al. [11]

Table 2
Preparation and characterization of the prepared stationary phases

| Stationary phase | Procedure | Chiral selector | Elemental analyses (%) | | | Amount of cellulose derivatives[a] |
|---|---|---|---|---|---|---|
| | | | C | N | H | |
| CSP1 | Non-reg[b] | CPC[c] | 7.68 | 0.46 | 0.34 | 12.2 |
| CSP2 | Non-reg | CPC | 8.21 | 0.58 | 0.30 | 14.6 |
| CSP3 | Reg[d] | CPC | 8.41 | 0.65 | 0.28 | 15.1 |
| CSP4 | Reg | CDMPC[e] | 9.58 | 0.69 | 0.34 | 16.4 |
| γ-MAPS | | | 2.33 | 0.00 | 0.44 | |

[a] Calculated from the results of elemental analyses (mass%).
[b] Non-regioselevctive.
[c] Cellulose phenylcarbamate.
[d] Regioselective.
[e] Cellulose 3,5-dimethylphenylcarbamate.

Table 3
Chromatographic data for separation of enantiomers on CSP1 to CSP3

| Racemate | Mobile phase | CSP1 | | | | CSP2 | | | | CSP3 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | $k'_1$ | $\alpha$ | Rs | $N_1$ | $k'_1$ | $\alpha$ | Rs | $N_1$ | $k'_1$ | $\alpha$ | Rs | $N_1$ |
| *trans*-Stilbene oxide | a | 0.43 | 1.23 | 1.21 | 40400 | 0.68 | 1.28 | 1.91 | 35600 | 0.48 | 1.26 | 1.59 | 36700 |
| Benzoin | b | 1.29 | 1.00 | – | – | 2.24 | 1.04 | – | – | 1.56 | 1.00 | | |
| 3-Butyl-phthalide | b | 0.84 | 1.11 | – | – | 1.35 | 1.14 | 1.21 | 28600 | 1.03 | 1.08 | – | – |
| Troger's base | c | 0.59 | 1.13 | – | – | 0.75 | 1.17 | 1.58 | 52500 | 0.68 | 1.08 | – | – |
| Warfarin | d | 0.80 | 1.41 | 3.62 | 45100 | 1.21 | 1.47 | 4.72 | 42900 | 0.92 | 1.38 | 3.84 | 56700 |
| Bendroflumethiazide | e | 0.78 | 1.26 | 2.31 | 53500 | 1.24 | 1.28 | 2.62 | 32200 | 0.81 | 1.22 | 1.70 | 34800 |
| Drug candidate A | e | 0.72 | 2.01 | 7.87 | 50600 | 1.07 | 2.10 | 10.2 | 55300 | 0.76 | 1.81 | 5.33 | 29900 |
| Drug candidate B | e | 1.54 | 1.22 | 2.84 | 52700 | 2.19 | 1.22 | 3.14 | 51800 | 1.73 | 1.13 | 1.47 | 34000 |
| Praziquantel | e | 1.16 | 1.26 | 2.53 | 42500 | 1.97 | 1.27 | 3.17 | 40000 | 1.23 | 1.25 | 2.29 | 35900 |

Mobil phases: (a) hexane/2-propanol (98/2); (b) hexane/2-propanol (90/10); (c) hexane/ethanol (90/10); (d) hexane/ethanol/acetic acid (80/20/0.2); and (e) hexane/ethanol/THF (70/30/4).

have reported the reaction of a mixture of 10-undecenoyl-3,5-dimethylphenylaminocarbonyl derivative cellulose with an allylsilica gel. Recently, Okamoto and co-works [17,18] have reported the preparation of CSPs by the polymerization of cellulose 3,5-dimethylphenylcarbamate with a vinyl monomer.

In our study, methacryloyl chloride was adopted as a spacer reagent to chemically fix cellulose phenylcarbamates onto vinylized silica gel. Before co-polymerization, methacryloyl chloride is expected to react with the hydroxyl groups on the glucose units, thus methacrylate groups would be introduced into cellulose phenylcarbamates. On the other hand, the vinylized silica gel (γ-MAPS) was prepared with the reaction of bare silica gel and γ-(trimethoxysilyl)propyl methacrylate. By this way, the radical co-polymerization reaction can be occurred between cellulose methacrylatephenylcarbamates and γ-MAPS with the presence of AIBN.

The results of elemental analyses for the prepared cellulose derivatives with methacrylate groups are shown in Table 1. It can be seen that the percentages of N and C were slightly varied with the amount of MC used and the procedures for preparation, which may indicate that those factors might affect the content of vinyl groups introduced onto cellulose derivatives as well. The data of elemental analyses for the prepared CSPs are listed in Table 2. As can be seen, the average value of mass percentage for chiral selectors bonded onto the silica is approximately 14.5%, which suggests that such a radical co-polymerization developed in this study could be efficiently achieved with the presence of AIBN. Although, the crosslinking of cellulose phenylcarbamate derivatives is possible during the polymerization reaction, the polymer may be still retained on the CSPs possibly due to the insolubility of the products of crosslinking. On the other hand, only if one of the vinyl groups on a cellulose chain is effectively reacted with the pore surface functionalities, the whole of molecular chain could be immobilized no matter how the rest go on.

In our experiments, cellulose derivatives containing the methacrylate can be prepared with non-regioselective and regioselective procedures, thus different cellulose derivative-based CSPs can be prepared with various derivatizing reagents. As listed in Table 2, CSP1–CSP3 are prepared with phenyl isocyanate as the derivatizing reagents and CSP4 was prepared with 3,5-dimethylphenyl isocyanate.

The prepared CSPs were evaluated by HPLC resolution of enantiomers under different mobile phases. As seen in Tables 3–5, the average plate number for the eluted enan-

Table 4
Chromatographic data for separation of enantiomers on CSP4 under classic mobile phase conditions

| Racemate | Mobile phase | $k'_1$ | $\alpha$ | Rs | $N_1$ (plates per meter) |
|---|---|---|---|---|---|
| *trans*-Stilbene oxide | a | 0.77 | 1.47 | 5.25 | 39500 |
| Troger's base | b | 0.95 | 1.16 | 3.72 | 72000 |
| Benzoin | b | 1.78 | 1.20 | 4.09 | 42000 |
| Warfarin | c | 0.86 | 1.56 | 5.97 | 25600 |
| Praziquantel | c | 2.02 | 1.25 | 3.46 | 20000 |
| α-DDB1 | c | 1.72 | 1.16 | 2.46 | 25000 |
| α-DDB3 | c | 3.74 | 1.12 | 2.29 | 23200 |
| Drug candidate A | d | 0.72 | 2.41 | 13.1 | 33400 |
| Drug candidate B | d | 1.65 | 1.17 | 2.89 | 30000 |
| Ranolazine | e | 2.43 | 1.87 | 6.85 | 12300 |
| Propranolol | f | 3.30 | 1.11 | 2.41 | 30200 |

Mobile phases: (a) hexane/2-propanol (98/2); (b) hexane/ethanol (95/5); (c) hexane/ethanol/acetic acid (80/20/0.5); (d) hexane/ethanol (50/50); (e) hexane/ethanol/*n*-caproic acid (75/25/0.1); and (f) hexane/ethanol/DEA (95/5/0.1).

Table 5
Chromatographic data for separation of enantiomers on CSP4 with the addition of THF and chloroform into the mobile phase

| Racemate | Mobile phase | $k'_1$ | $\alpha$ | Rs | $N_1$ (plates per meter) |
|---|---|---|---|---|---|
| *trans*-Stilbene oxide | a | 0.57 | 1.58 | 6.56 | 56800 |
| Benzoin | a | 2.22 | 1.31 | 6.05 | 34600 |
| | b | 3.64 | 1.38 | 5.76 | 23300 |
| Troger's base | b | 1.51 | 1.16 | 3.72 | 38100 |
| Praziquantel | c | 1.61 | 1.53 | 6.00 | 22100 |
| Drug candidate A | c | 1.97 | 3.07 | 26.3 | 40300 |
| Ranolazine | d | 2.55 | 1.31 | 3.11 | 9000 |
| Drug candidate B | c | 4.92 | 1.12 | 2.97 | 36300 |
| | e | 3.85 | 1.20 | 4.41 | 35800 |
| α-DDB1 | c | 0.89 | 1.09 | – | – |
| | e | 0.37 | 1.13 | – | – |
| α-DDB2 | c | 1.24 | 1.17 | 2.73 | 34600 |
| α-DDB3 | c | 1.62 | 1.31 | 4.34 | 25600 |

Mobile phases: (a) hexane/chloroform (95/5); (b) hexane/THF (95/5); (c) hexane/THF/ethanol/acetic acid (80/20/5/0.1); (d) hexane/THF/ethanol (70/20/10); and (e) hexane/chloroform/ethanol/acetic acid (80/20/5/0.1).

tiomers on the prepared CSPs is about 30,000 plates per meter. Such a high column efficiency was generally observed for the test enantiomers on the newly prepared CSP, which suggests that the newly developed radical co-polymerization reaction is perfect for preparation of polysaccharide-based CSPs.

### 3.2. Effect of the amount of methacrylolyl chloride on resolution

In our study, the methacrylate groups on cellulose derivatives were introduced via the bifunctional reagent of methacrylolyl chloride. For the non-regioselectively fixed procedures, cellulose was simultaneously reacted with a mixture of phenyl isocyanate and methacrylolyl chloride, then the cellulose derivatives was immobilized to silica matrix through the radical co-polymerization between methacrylate groups. In this case, methacrylolyl chloride and phenyl isocyanate are competitively reacted with hydroxyl groups of cellulose. Thus, the amount of methacrylolyl chloride used might affect the surface coverage of chiral selector on silica matrix and play a role on chiral resolution.

The effect of the amount of methacrylolyl chloride on Enantio-separations was investigated on the non-regioselectively prepared CSPs. As described in Table 2, CSP1 and CSP2 were prepared with the same chiral selector by the non-regioselective procedures except for different amounts of methacrylolyl chloride used for preparation. The chromatographic results of Enantio-separations on the two CSPs are listed in Table 3. As is seen, insignificant difference was observed for the $\alpha$ values on CSP1 and CSP2, while the resolution (Rs) on CSP2 was generally higher than those on CSP1. For example, for the resolution of warfarin, as shown in Fig. 3, the magnitude of Rs value on CSP2 (II) was greater than that on CSP1 (I). Thus it is indicated that the amount of methacrylolyl chloride used may affect the enantiomeric resolution on the prepared CSPs.

### 3.3. Comparison of non-regioselective and regioselective procedures

In our experiments, the chemical immobilization was carried out with non-regioselective and regioselective procedures. A direct comparison was performed on the CSPs prepared with the two methods. As listed in Table 2, CSP3 was prepared with the regioselective procedures and CSP2 was obtained with the non-regioselective procedures. The chromatographic results for separation of enantiomers on the two CSPs are listed in Table 3. As can be seen, generally there is no significant difference of the chiral separations on the two phases except that the values of $\alpha$ and Rs on CSP3 were somewhat little lower than those on CSP2 for some test race-

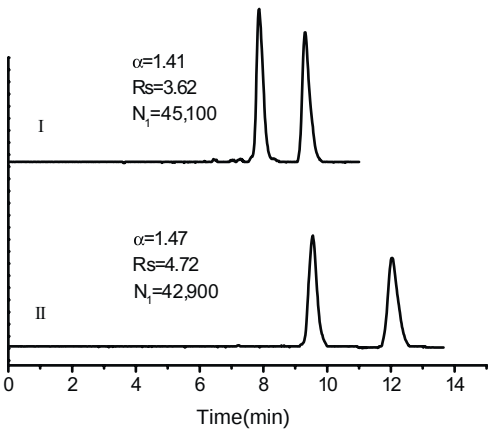


Fig. 3. Chromatograms for separation of warfarin on CSP1 (I) and CSP2 (II). Chromatographic conditions are described in Table 3.

mates. The theoretical plate numbers for the eluted enantiomers on the two phases are over 30,000 plates per meter. The above results may suggest that methacrylolyl chloride is preferred to react with the primary hydroxyl groups on cellulose either in non-regioselective or regioselective procedures. Thus, no significance of chiral resolving power was observed on the non-regioselectively prepared CSP2 and regioselectively prepared CSP3.

On the other side, the regioselective procedures are rather complicated due to the introduction of the protecting group and it takes about 6 days to prepare one column. While for the non-regioselective procedures, the spacer reagent of methacrylolyl chloride and derivatizing reagent were simultaneously reacted with the cellulose. In this case, the CSP can be prepared within 2 days. Thus the non-regioselective procedures seem to be more useful for the rapid preparation of cellulose-based CSPs.

### 3.4. Separation of enantiomers with addition of THF and chloroform in the mobile phase

In this study, CSP4 was prepared by chemically bonding cellulose 3,5-dimethylphenylcarbamte onto silica with the regioselective procedures, as listed in Table 2. The chromatographic evaluation on CSP4 is presented in Table 4 with the classic mobile phases of hexane/alcohols. Generally, most of the test enantiomers were separated well on the CSP4 with high column efficiency. As can be seen, the average plate numbers of the eluted enantiomers was about 30,000 plates per meter. Enantio-separations of Troger's base on CSP4 was a representative instance, as shown in Fig. 4, the plate number of the firstly eluted peak was even high up to 72,000 plates per meter, which was rarely reported in literature for HPLC resolution of enantiomers.

As we know, the commercially available polysaccharide-based chiral columns, which are prepared by physically coating polysaccharide derivatives onto silica matrices, are limited to be used because of the solubility of polysaccharide derivatives in a number of solvents [7]. In our experiments, cellulose phenylcarbamates are chemically immobilized onto silica matrix with the radical co-polymerization reaction. Thus the above problem can be solved and the limitation of solvents used as mobile phases for chromatographic separations is greatly avoided. Therefore, some organic solvents such as THF and chloroform, which can lead to the dissolution of cellulose derivatives and generally cannot be adopted on the coated type of CSPs, have a possibility to be used as a composition in the mobile phase for chiral separations on the CSPs prepared in this study. The chromatographic data for separation of enantiomers on CSP4 with the addition of THF and chloroform into the mobile phase are listed in Table 5.

As is seen, 10 enantiomers were evaluated with various mobile phase conditions in this case. Again, high column efficiency for the eluted enantiomers was observed. The average plate number for the firstly eluted peaks exceeded 30,000 plates per meter. In addition, it was observed that the enantioseletivities of some racemates were improved to various degrees with the addition of THF or chloroform into the mobile phase. For example, as shown in Fig. 5, the $\alpha$ value for separation of *trans*-stilbene oxide under mobile phase (I) with the addition of chloroform was higher than that obtained under mobile phase (II) with the addition of 2-propanol. The plate number of the eluted enantiomers was also enhanced at the same time. Another representative example is presented in Fig. 6 for the separation of benzion, again the enantioselectivity was increased about 9 and 15%, respectively, with the addition of THF (II) and chloroform (III) into the mobile phases.

It was also observed that the enantioselectivities were influenced by the concentration of THF in the mobile phase.

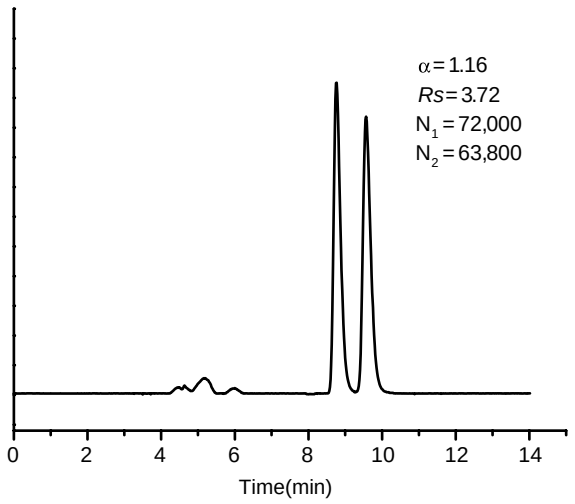


Fig. 4. Chromatogram for separation of Troger's base on CSP4. Chromatographic conditions are described in Table 4.

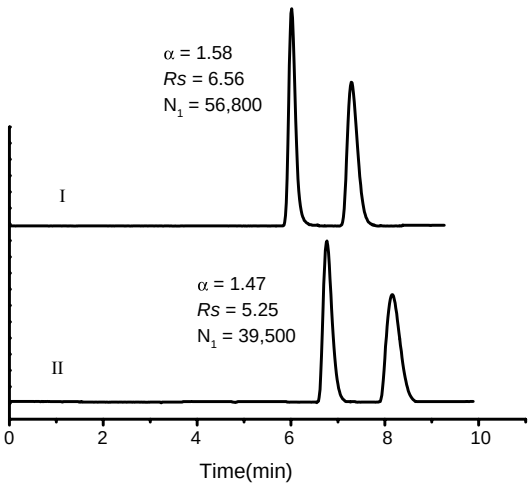


Fig. 5. Chromatograms for separation of *trans*-stilbene oxide on CSP4. Mobile phases: (I) hexane/chloroform (95/5) and (II) hexane/2-propanol (98/2).

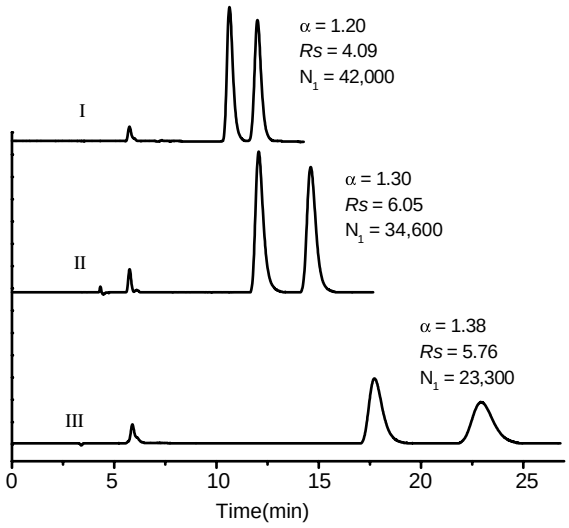


Fig. 6. Chromatograms for separation of benzoin on CSP4. Mobile phases: (I) hexane/ethanol (95/5); (II) hexane/chloroform (95/5); and (III) hexane/THF (95/5).

As presented in Fig. 7, the $\alpha$ value for separation of praziquantel (a) was increased about 15% with the concentration of THF from 5% (I) to 20% (II) in the mobile phase. And the $\alpha$ value for separation of α-DDB2 (b) was increased about 8% under the same case.

Although chiral recognition for enantiomers on the prepared CSP can be improved in various degrees by mobile phase compositions of THF and chloroform, it would not work at any cases. For example, as listed in Tables 4 and 5, it can be seen that the magnitude of $\alpha$ values for separation of α-DDB1 and ranolazine was decreased from 1.16 to 1.09 and from 1.87 to 1.31, respectively, with the addition of THF and chloroform into the mobile phases.

Separation of enantiomers on the prepared CSPs can be greatly affected by addition of THF and chloroform in the mobile phases, which may indicate that chiral interactions between the solute and the CSP might be changed with the presence of THF and chloroform. Such a phenomenon has also been reported previously [7,9], and it was ascribed to some conformational changes of the polysaccharides because polysaccharide derivatives may be soluble or swollen under the presence of chloroform and THF [9].

Above discussion demonstrated that elimination of solubility of cellulose derivatives on the bonded-type CSPs represents advantages in the search for new applications on these kinds of CSPs. That is to say, some racemates which are not or poorly separated on coated type of polysaccharide-based CSPs might be efficiently resolved on bonded-type CSPs with addition of solvents such as THF and chloroform in the mobile phase.

### 3.5. Reproducibility

A series of CSPs (columns 1–4) were prepared with the non-regioselective immobilization of cellulose phenylcarbamate under the same conditions in order to evaluate the batch-to-batch reproducibility for this radical copolymerization reaction. The chromatographic results for separation of four enantiomers are described in Table 6. As can be seen from Table 6, the respective value of the capacity factor for the firstly eluted enantiomer is very close on the series CSPs except that obtained from the column 3, and no marked difference was observed for the enantioselectivities on CSPs prepared with different batches. Also, the column efficiency for the test enantiomers on columns 1–4 approximately exceeded 30,000 plates per meter.

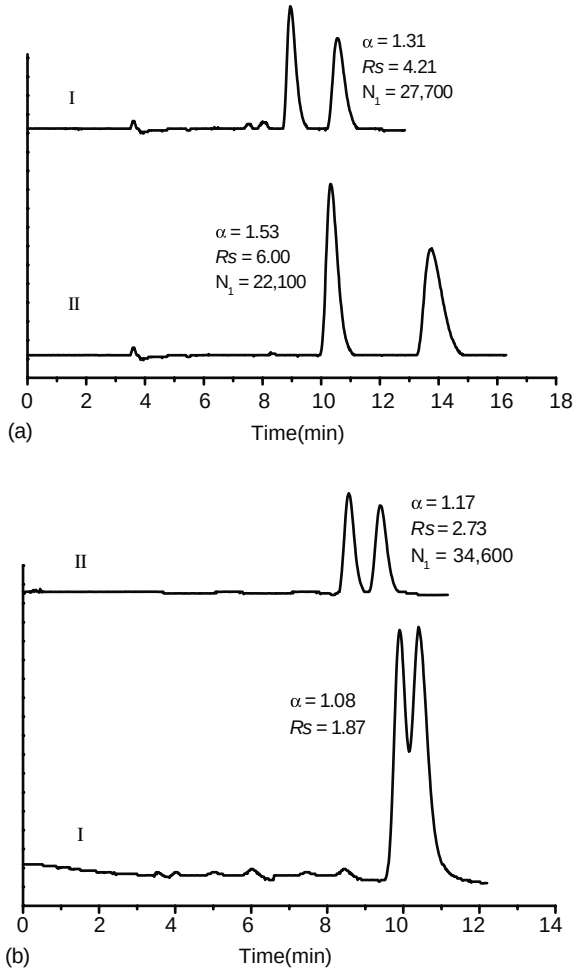


Fig. 7. Chromatograms for separation of praziquantel (a) and α-DDB2 (b) on CSP4 with the addition of THF in the mobile phase. Mobile phases: (I) hexane/ethanol/THF/acetic acid (80/20/5/0.1) and (II) hexane/ethanol/THF/acetic acid (80/5/20/0.1).

The run-to-run reproducibility for separation of enantiomers on the prepared CSPs was also investigated with decades of consecutive injections under the same mobile phase condition. The chromatographic results are described in Table 7. As can be seen, the magnitude of R.S.D. values for $t_1$ and $t_2$ is less than 0.6% on CSP3 and CSP4 for several test racemates. The prepared CSPs are still very stable even under the mobile phase condition containing 20% chloroform.

Table 6
Chromatographic results for batch-to-batch reproducibility

| Racemate | Mobil phase | Column 1 | | | Column 2 | | | Column 3 | | | Column 4 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | $k'_1$ | $\alpha$ | $N_1$[a] | $k'_1$ | $\alpha$ | $N_1$ | $k'_1$ | $\alpha$ | $N_1$ | $k'_1$ | $\alpha$ | $N_1$ |
| *trans*-Stilbene oxide | a | 0.24 | 1.35 | 27.9 | 0.26 | 1.24 | 32.1 | 0.38 | 1.30 | 34.5 | 0.27 | 1.23 | 40.4 |
| Troger's base | b | 0.47 | 1.12 | – | 0.48 | 1.10 | – | 0.57 | 1.14 | – | 0.48 | 1.13 | – |
| Bendroflumethiazide | c | 0.74 | 1.27 | 47.3 | 0.75 | 1.24 | 34.0 | 1.09 | 1.24 | 33.5 | 0.77 | 1.26 | 53.5 |
| Warfarin | d | 0.64 | 1.41 | 49.7 | 0.66 | 1.34 | 33.6 | 0.93 | 1.39 | 37.3 | 0.69 | 1.41 | 45.1 |

Mobile phases: (a) hexane/2-propanol (98/2); (b) hexane/2-propanol (90/10); (c) hexane/ethanol/THF (70/30/4); and (d) hexane/ethanol/acetic acid (80/20/0.2).
[a] $1 \times 10^3$ plates per meter.

Table 7
Chromatographic results for run-to-run reproducibility

| CSP | Mobile phase | Racemate | Injection times (units) | R.S.D.[a] (%) | |
|---|---|---|---|---|---|
| | | | | $t_1$ | $t_2$ |
| CSP3 | a | Praziquantel | 20 | 0.54 | 0.53 |
| | a | Troger's base | 15 | 0.16 | 0.11 |
| CSP4 | a | Ranolazine | 20 | 0.27 | 0.37 |
| | a | Praziquantel | 15 | 0.31 | 0.53 |
| | b | Benzoin | 20 | 0.51 | 0.49 |

[a] $t_1$ and $t_2$ are the retention time for the first and second eluted enantiomers. Mobile phases: (a) pure ethanol; and (b) hexane/chloroform (80/20).

Above results indicate that the cellulose derivative-based CSPs prepared in this study are very stable for HPLC separation of enantiomers, and the newly developed radical co-polymerization reaction can be reproducible.

## 4. Conclusion

The bonded-type of CSPs with cellulose derivatives can be efficiently synthesized with the radical co-polymerization reaction via methacrylates under presence of AIBN. The CSPs can be prepared through non-regioselective and regioselective procedures with various derivatizing reagents. The preparation of the chiral materials is reproducible and considerably high column efficiency is generally observed for resolution of the test enantiomers. In addition, the prepared CSPs are very stable with the addition of some solvents such as THF and chloroform into mobile phases, which makes it possible to extend the application range on the newly prepared CSPs for separation of enantiomers.

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