Synthesis of covalently bonded cellulose derivative chiral stationary phases with a bifunctional reagent of 3-(triethoxysilyl)propyl isocyanate

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Abstract

A bifunctional reagent of 3-(triethoxysilyl)propyl isocyanate (TEPI) was initially adopted as a spacer reagent to prepare the bonded types of chiral stationary phases (CSPs) with cellulose derivatives. The silica-based CSPs were chemically prepared with non-regioselective and regioselective approaches and their chiral resolving capabilities were evaluated in terms of HPLC resolution of test enantiomers. It was observed that the chiral recognition capabilities of the non-regioselectively prepared CSPs were influenced by the amount of TEPI used. And also, the regioselectively prepared CSP generally showed a slightly higher resolution power than the non-regioselectively prepared CSP, while the non-regioselective procedures were highly advantageous to rapid preparation. In addition, chiral recognition of the prepared CSPs was affected by the properties of the used silica matrices.
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1. Introduction

Chiral stationary phases (CSPs) based on cellulose derivatives play a big role in enantioseparations [1]. Polysaccharide derivatives coated onto silica matrix show powerful chiral resolving abilities and these CSPs are commercially available [2–7]. Although a very large range of enantiomers have been separated on these coated CSPs, the main drawback of these phases is related to the solubility of chiral selectors in a number of solvents, thus the solvents used as the mobile phases on coated phases are limited [8]. In order to overcome this problem, synthesis of polysaccharide-based CSPs with chemical procedures has attracted great attention nowadays. In this way, the number of solvents used as the mobile phase is greatly extended. Up to now, various methods have been successfully developed to prepare the bonded-type phases reported by Okamoto and co-workers [9–11], Minguillón et al. [12–14], and others [8]. Recently, we have successfully synthesized a composite type of CSP by chemically bonding two different cellulose derivatives onto a silica matrix [15].

In this study, the main objective is to develop a novel method to synthesize bonded types of cellulose derivative chiral stationary phases. For this goal, a

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bifunctional reagent of 3-(triethoxysilyl)propyl iso-cyanate (TEPI), on which the isocyanate groups are expected to react with the hydroxyl groups of cellulose derivatives and the ethoxysilyl groups to react with the silanol groups, was adopted as the spacer reagent to chemically fix cellulose derivatives onto silica matrix. Two different procedures were adopted for the preparation of CSPs and their chiral resolution capabilities were investigated.

2. Experimental

2.1. Chemicals

Microcrystalline cellulose was obtained from Serva (Heidelberg, Germany). Silica I (Kromasil, 5 μm, 200 Å, 200 m^2/g) was purchased from Akzo Noble (Nacka, Sweden) and Silica II (Nucleosil, 7 μm, 300 Å, 100 m^2/g) from Macherey–Nagel (Düren, Germany). Trityl chloride, 3,5-di-methylphenyl isocyanate and 4-methylphenyl chlo-ride were obtained from Sigma–Aldrich (Gilling-ham, UK). Phenyl isocyanate was purchased from Acros (NJ, USA). TEPI was obtained from Aldrich (Milwaukee, WI, USA). Other reagents are of ana-lytical grade.

The racemic compounds of *trans*-stilbene oxide, benzoin, warfarin, praziquantel, bendroflumethiazide, alprenolol, metoprolol, propranolol 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 the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). α-Dimethyl dicarboxyl biphenyl derivative (α-DDBD) was obtained from the Lanzhou Institute of Chemical Physics. 3-Butyl-phthalide was obtained from CSPC Pharmaceutical Technology (Shijiazhuang, China). The molecular structures of these solutes are presented in Fig. 1.

2.2. Preparation of CSPs with non-regioselective procedures

The non-regioselective procedures are shown in Fig. 2. The dried cellulose (0.8 g) was dispersed in pyridine (30 ml) containing a mixture of derivatizing reagent (3.0 ml) and various amounts of TEPI, and

was allowed to react at 90 °C for 10 h. Then the cooled solution was poured into a large flask and isolated as the fraction insoluble in methanol. Thus cellulose phenylcarbamate derivatives (I, II and V) containing triethoxypropyl silane were obtained as described in Table 1.

The modified cellulose derivative (1.0 g) was coated onto silica (2.0 g) and dispersed into a mixture of toluene (10.0 ml) and pyridine (4.0 ml), then the reaction continued to proceed at about 95 °C for another 8 h. Thus CSPs were obtained by centrifugation and washed completely with pyridine, tetrahydrofuran (THF) and methanol, respectively, in order to remove the free cellulose derivatives. Re-peating above procedures, CSP1, CSP2, CSP4 and CSP6 were prepared with corresponding chiral selec-tors and chromatographic matrices as listed in Table 2.

2.3. Preparation of CSPs with regioselective procedures

2.3.1. Synthesis of cellulose 2,3-bisderivatives

Procedures for synthesis of cellulose 2,3-bis-derivatives, which were initially developed by Yashima et al. [10], are shown in Fig. 3. Dried microcrystalline cellulose (3.0 g) was allowed to react with triphenylmethyl chloride (10.5 g) in pyridine (60.0 ml) at about 90 °C for 24 h, which can react only with the primary hydroxyl groups at the 6-position of the glucose units to form trityl ethers. Then various derivatizing reagents (phenyl iso-cyanate and *p*-methylbenzoyl chloride, 10.0 ml) were added, respectively, to form carbamate or benzoate residues with the hydroxyl groups at the 2- and 3-positions. After that, the trityl groups were removed by suspending in methanol (300 ml) con-taining a small amount of hydrochloric acid at room temperature for 24 h. Thus cellulose 2,3-bis-(phenylcarbamate) (CPC) and cellulose 2,3-bis-(methylbenzoate) (CMB) were obtained, respective-ly, after filtration and washing with a large amount of methanol.

2.3.2. Regioselectively bonded at the 6-positions of the glucose units

The regioselective procedures are shown in Fig. 4. Cellulose 2,3-bisderivative was dissolved in pyridine

trans-Stilbene oxide   Benzoin   3-Butyl-phthalide   Ranolazine

Troger's base   Warfarin   Praziquantel   Bendroflumethiazide

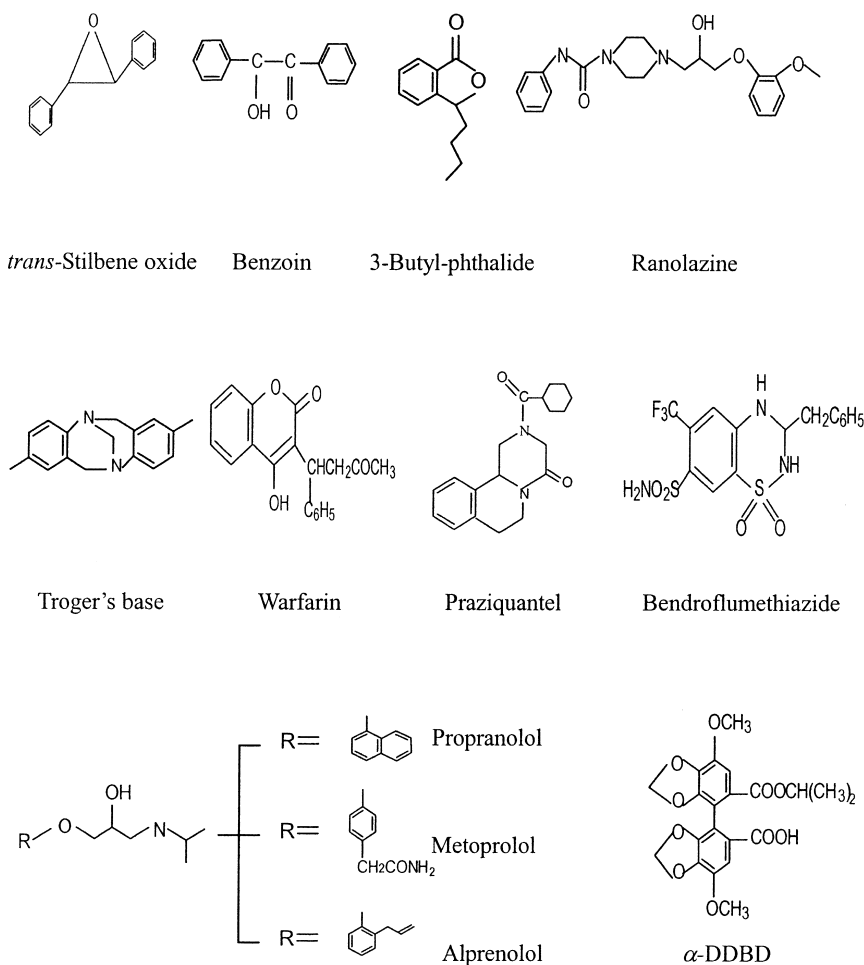R= Propranolol

R= Metoprolol

R= Alprenolol

α-DDBD

Fig. 1. Molecular structures of the test enantiomers.

(30 ml) containing an excessive amount of TEPI, and was allowed to react at 90 °C for 10 h. After that, the product was isolated as the fraction insoluble in methanol. Thus, cellulose derivatives III and IV were prepared containing triethoxypropyl silane at the 6-positions. Then the modified cellulose derivative (1.0 g) was coated onto silica matrix (2.0 g), and dispersed into a mixture of toluene (10.0 ml) and pyridine (4.0 ml). The reaction was continued to proceed at about 95 °C for another 8 h. Finally, the

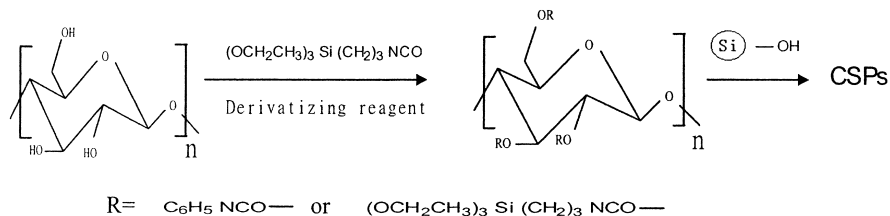R= C_6H_5 NCO— or $(OCH_2CH_3)_3$ Si $(CH_2)_3$ NCO—

Fig. 2. Procedures for synthesis of the non-regioselectively bonded CSPs with TEPI as the spacer reagent.

Table 1
Characterization of cellulose phenylcarbamate derivatives prepared by non-regioselective and regioselective procedures

Cellulose derivative*	Procedure	Amount of TEPI (μl)	Elemental analyses		
			C (%)	N (%)	H (%)
I	Non-regioselective	150	54.6	6.75	3.89
II	Non-regioselective	300	58.8	7.50	5.32
III	Regioselective	300	59.4	9.63	5.12
IV	Regioselective	300	55.2	3.21	4.65
V	Non-regioselective	300	59.9	7.56	5.41

* I and II were prepared with the reaction of cellulose and the mixture of TEPI and phenyl isocyanate; III was prepared with the reaction of cellulose 2,3-bis(phenylcarbamate) and TEPI; IV was prepared with the reaction of cellulose 2,3-bis(4-methylbenzoate) and TEPI; V was prepared with the reaction of cellulose and the mixture of TEPI and 3,5-dimethylphenyl isocyanate.

Table 2
Types of the prepared CSPs and the data of elemental analyses

CSP	Silica	Cellulose derivative	Ratio[γ] (mmol/g)	Procedure[ϕ]	Elemental analyses			Amount of cellulose derivative[λ]
					N (%)	C (%)	H (%)	
CSP1	Silica I	I	0.58	A	0.38	2.28	0.34	4.16
CSP2	Silica I	II	0.97	A	0.42	2.67	0.37	4.87
CSP3	Silica I	III	0.97	B	1.22	7.28	0.67	13.3
CSP4	Silica II	II	0.97	A	0.50	3.82	0.29	6.96
CSP5	Silica II	IV	0.97	B	0.33	2.12	0.31	3.54
CSP6	Silica II	V	0.97	A	0.38	3.02	0.37	5.26

[γ] Millimol of the spacer reagent vs. the mass of cellulose derivatives.
[ϕ] A, Non-regioselective fixation; B, regioselective fixation.
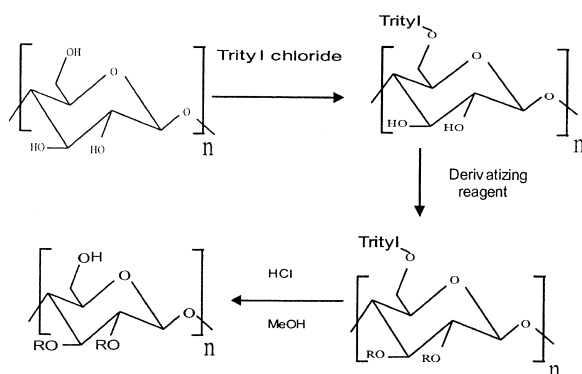[λ] The amount of cellulose derivatives onto silica gel calculated from C (%) of CSPs (mass %).

product was completely washed by pyridine, THF and methanol, respectively. Thus CSP3 and CSP5 were obtained with CPC and CMB as the chiral

R = C_6H_5NHCO- (CPC);   R = $CH_3C_6H_4$CO- (CMB)

Fig. 3. Scheme for preparation of cellulose 2,3-bisderivatives.

selectors by repeating the above procedures, respectively.

2.4. Apparatus and chromatography

The high-performance liquid chromatography (HPLC) experiments were performed with a Waters 510 pump (Waters, Milford, MA, 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 in pure methanol and packed into stainless steel columns (150×4.6 mm I.D.) by a slurry packing technique. Enantioseparations were performed with a flow-rate of 0.5 ml/min at ambient temperature except when otherwise stated. The mobile phases were filtered and sonicated prior to use. The void time of the columns were determined by 1,3,5-tri-*tert*.-butylbenzene with hexane–ethanol–acetic acid (70:30:0.2, v/v) as the mobile phase. All of the solutes were detected at 254

Fig. 4. Procedures for synthesis of the regioselectively bonded CSPs with TEPI as the spacer regent.

3. Results and discussion

3.1. Characterization of cellulose derivatives and the prepared CSPs

The prepared cellulose 2,3-bisderivatives were characterized by Fourier transform (FT) IR spectrum. The results are described below. CPC: 3320 (NH), 1725 (C=O), 1600 and 1525 (C_6H_5), cm^{-1}; CMB: 1725 (C=O), 1600 and 840 (C_6H_5), cm^{-1}. In addition, the peaks due to hydroxyl groups at around 3500 cm^{-1} were found on FT-IR spectrum for the cellulose 2,3-bisderivatives. These results proved that the cellulose derivatives were obtained successfully with corresponding derivatizing reagents and hydroxyl groups are available on the synthesized cellulose 2,3-bisderivatives.

In addition, cellulose derivatives containing triethoxysilyl groups were prepared with non-regioselective and regioseletive procedures before chemical bonding to the silica gel. In order to characterize the cellulose derivatives from the quantitative point of view, elemental analyses have been carried out and the results are described in Table 1. In addition, the prepared derivatives were characterized by ^{1}H nuclear magnetic resonance (NMR) spectroscopy. The signals of phenyl groups (δ 6.6–7.6, ppm), which were assigned to the amount of cellulose phenylcarbamate or benzoate in the cellulose derivatives, were observed in ^{1}H NMR. Unfortunately, the signals due to the propyltriethoxysilane in the derivatives are significantly low. No visible differences are observed for the ^{1}H NMRs to all the derivatives.

This might because that the solubility of these cellulose derivatives in pyridine is not high enough for the ^{1}H NMR experiments (300 MHz, pyridine-d_5). And also, the ethoxy groups might be lost during the preparation of the derivatives by heating or by simple hydrolysis resulting in poor signals [16].

The prepared CSPs were also characterized by FT-IR spectrum. The peaks due to the carbonyl group at around 1725 cm^{-1} and phenyl group at around 1600, 1525 cm^{-1} were observed, respectively, which indicated that cellulose derivatives were successfully fixed to silica surface. Elemental analyses of the bonded CSPs were carried out, and the obtained results are listed in Table 2. It can be seen that the mass percentage of the cellulose derivatives onto the silica gel was somewhat lower than those in previous reports [10,12]. It may be possibly attributed to the short carbon chain of the bifunctional reagent of TEPI used in our experiments, which makes the ethoxysilyl groups on the cellulose derivatives not to be accessible to the inner surface of the silica gel with small pore size of 200 or 300 Å. In addition, the propyltriethoxy groups might be lost during the preparation of the derivatives as discussed above [17], which might also result in poor chiral moieties on the silica gel.

3.2. Effect of the amount of TEPI on enantioseparations

In our experiments, a bisfunctional reagent of TEPI, which contains two different functional groups of ethoxysilyl and isocyanate, was used as a spacer reagent to immobilize cellulose derivatives onto silica matrix. The isocyanate groups are expected to react with the hydroxyl groups of cellulose derivatives, while the ethoxysilyl groups to react with the silanol groups. Two different approaches were used

to prepare the CSPs with this spacer reagent. One of them is the regioselective fixation, that is, the chiral selector was chemically immobilized to silica matrix through the 6-positions (the primary hydroxyl groups) of the glucose units. As shown in Fig. 4, cellulose 2,3-bisderivative was completely reacted with TEPI at the 6-positions prior to bonding onto silica matrix. The surface coverage of chiral selector can be to a maximum value reacted with an excessive amount of TEPI in this way. But for the non-regioselectively fixed procedures, cellulose was simultaneously reacted with a mixture of derivatizing reagent and TEPI, and was immobilized to silica matrix through the ethoxysilyl groups on the modified cellulose derivative. In this case, the isocyanate groups of TEPI and derivatizing reagent are competitively reacted with the hydroxyl groups of cellulose. Thus, the amount of TEPI used might affect the surface coverage of chiral selector on silica matrix.

The effect of the amount of TEPI on enantio-separations was investigated for the non-regioselectively prepared CSPs with CPC as the chiral selector. As described in Table 2, CSP1 and CSP2 were prepared with 0.58 and 0.97 mmol of TEPI by the non-regioselective method. The chromatographic results of enantioseparations for the test enantiomers on the two CSPs are listed in Table 3. As is seen, CSP2 generally showed higher chiral resolving capabilities than CSP1. That is to say, enantioselectivity increased with the increasing amount of TEPI used. For example, for the separation of warfarin, as
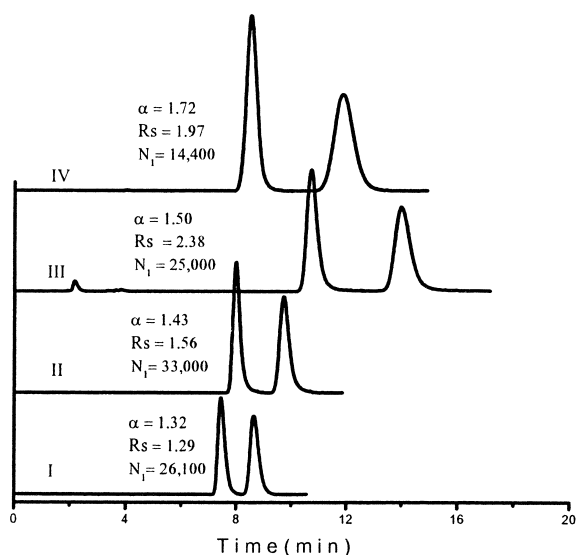

Fig. 5. Chromatographic resolution of warfarin on CSP1 (I), CSP2 (II), CSP3 (III) and CSP4 (IV). Chromatographic conditions are described in Table 3.

shown in Fig. 5, the magnitude of α and R_s values on CSP2 (II) was greater than those on CSP1 (I). The higher chiral recognition power of CSP2 might attribute to the fact that the surface coverage of the chiral selector was improved with the increasing amount of the spacer reagent used. It can be supported from the data results of the elemental analyses of the prepared CSPs. As described in Table 2, the magnitude of percentages of C and N of CSP2 was

Table 3
Chromatographic data for resolution of enantiomers on CSPs prepared with CPC as chiral selector

Racemate	Mobile phase	CSP1			CSP2			CSP3			CSP4		
		k_1'	α	R_s	k_1'	α	R_s	k_1'	α	R_s	k_1'	α	R_s
trans-Stilbene oxide	a	0.55	1.11	0.44	0.19	1.36	0.60	0.53	1.35	1.28	0.27	1.43	0.98
Troger's base	a	1.36	1.04	–	1.50	1.10	–	1.50	1.14	0.70	1.52	1.09	0.65
3-Butyl-phthalide	a	1.12	1.04	–	1.25	1.09	0.77	2.19	1.08	0.81	1.68	1.14	0.84
Benzoin	b	2.02	1.05	–	2.44	1.06	0.69	3.79	1.10	0.71	3.64	1.04	–
Warfarin	c	0.97	1.32	1.29	1.00	1.43	1.56	1.60	1.50	2.38	1.21	1.72	1.97
Praziquantel	d	1.22	1.24	0.99	1.49	1.28	1.21	3.06	1.33	1.76	2.40	1.28	1.31
Bendroflumethiazide	d	1.32	1.21	1.29	1.33	1.31	1.16	3.84	1.23	1.26	1.82	1.38	1.56
Drug candidate A	e	2.43	1.17	1.12	2.93	1.15	1.09	4.52	1.15	1.04	3.89	1.23	1.11
Drug candidate B	e	0.61	1.83	2.55	0.71	2.10	2.13	1.38	2.05	4.55	1.03	2.55	3.34
α-DDBD	e	0.88	1.13	0.73	1.09	1.17	0.83	2.64	1.17	1.10	1.87	1.22	0.98

* The R_s values were calculated based on the bottom of peak width of the eluted enantiomers, respectively. Mobile phases: (a) hexane–ethanol (98:2), (b) hexane–tetrahydrofuran (THF)–2-propanol (96:4:2), (c) hexane–ethanol–acetic acid (85:15:0.2), (d) hexane–ethanol–THF–acetic acid (80:20:4:0.2), (e) hexane–ethanol–acetic acid (70:30:0.2).

greater than those of CSP1, which indicated a higher chiral moiety was acquired on CSP2.

Although the surface coverage could be enhanced with the increasing amount of spacer reagent used, the solubility of the intermediate (the product of the first step in the non-regioselective procedures) in THF or pyridine constitutes a problem with the higher amount of TEPI used according to our further investigation. For instance, when the ratio was added up to 1.54 mmol/g (millimol of the spacer reagent vs. the mass of cellulose), the solubility of the modified cellulose derivative in THF or pyridine was considerably low. For this reason, the residues in the solvent were difficult to completely remove from the CSP, thus the obtained CSP presented an extremely high pressure and even cannot be properly packed.

3.3. Comparison of regioselective and non-regioselective procedures

As described in Table 2, CSP1 and CSP2 were prepared with the non-regioselective procedures, while CSP3 was obtained with the regioselective method. These phases were synthesized based on the same chiral selector of cellulose phenylcarbamate modified by phenyl isocyanate. Table 3 summarizes the chromatographic results of enantioseparations on these phases. As can be seen, the regioselectively prepared CSP3 generally exhibited slightly higher chiral resolution ability than the non-regioselectively prepared phases of CSP1 and CSP2. For instance, as plotted in Fig. 5, separation of warfarin on CSP3 (III) were better than that on both CSP1 (I) and CSP2 (II). Separation of enantiomer *trans*-stilbene oxide was another typical example, as shown in Fig. 6, better resolution was also achieved on CSP3 (II) compared to CSP2 (I). But for some racemates, such as enantiomer bendroflumethiazide, as presented in Fig. 7, peak shape of the eluted enantiomers on the non-regioselectively prepared phase of CSP2 was sharper than that on the regioselectively prepared phase of CSP3 in some degrees. That is, the plate numbers of the first eluted peak (N_1) was 20 700 plates/m on CSP2 (I), while it was only 14 600 plates/m on CSP3 (II).

On the other hand, it is clearly observed that the procedures of the non-regioselective method were markedly simple than those of the regioselective
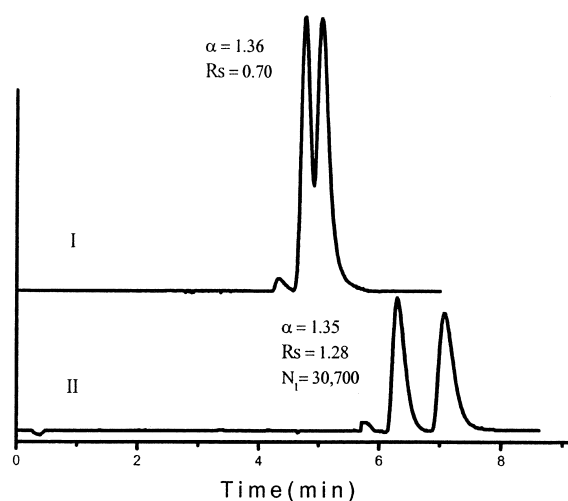

Fig. 6. Chromatographic resolution of *trans*-stilbene oxide on CSP2 (I) and CSP3 (II). CSP2 and CSP3 were prepared with non-regioselective and regioselective procedures, respectively. Chromatographic conditions are described in Table 3.

way. As shown in Figs. 3 and 4, the regioselective procedures were very complicated and it almost took 5 days to obtain one type of CSP. While for the non-regioselective fixation as shown in Fig. 2, CSP can be easily prepared within 24 h after two-step chemical reaction, and this is one of the most convenient processes to prepare the bonded type of cellulose-based CSPs according to the reported documents. So the non-regioselective procedures showed
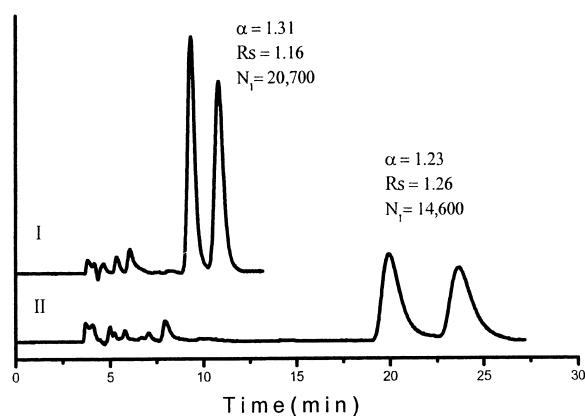

Fig. 7. Chromatographic resolution of bendroflumethiazide on CSP2 (I) and CSP3 (II). CSP2 and CSP3 were prepared with non-regioselective and regioselective procedures, respectively. Chromatographic conditions are described in Table 3.

great advantage from this point although it might sacrifice a little chiral resolution power compared to the regioselective fixation.

The chiral discrimination between the two kinds of phases might be caused from the following aspects. One is the difference of their steric structures of chiral selectors. For the regioselective method, cellulose was chemically fixed at the 6-positions of the glucose units, while for the non-regioselective fixation, cellulose derivative was bonded at 2,3- or 6-positions. It was reported that among the carbamate groups, those at the 2- and 3-positions seem to be more responsible for effective chiral recognition in the polysaccharide derivative-based CSPs [10]. Thus the regular arrangement of carbamate groups at the 2- and 3-positions may be more important to generate a higher enantioselectivity. The other possible reason is that the ethoxysilyl groups modified on the 6-positions are more accessible to the silanol groups compared to those located in the 2,3-positions because of the reduction of steric hinderance. Thus, the chiral moiety of CSP3 is higher than that of CSP2. The results of elemental analyses were also demonstrated that the surface coverage on CSP3 was higher than that on CSP2. As a consequence, the regioselectively prepared CSP shows a higher resolution power than the non-regioselectively prepared CSP.

During our experiments, we tried to regioselectively immobilize cellulose derivatives to silica matrix through the 2,3-positions of the glucose unites, but the solubility of the intermediate (cellulose derivatives regioselectively modified by TEPI at the 2,3-positions) is very poor in THF or pyridine, thus it is difficult to remove the free residues from the CSP. For this reason, the obtained CSP exhibit a higher pressure and significantly low performance for the test enantiomers.

3.4. Influence of silica matrix on enantioseparations

In our study, two kinds of silica with different pore and particle diameter were used for the preparation of CSPs in order to investigate the influence of properties of chromatographic matrix on enantioseparations. As described in Table 2, CSP2 and CSP4 were prepared with the non-regioselective procedures under the same experimental conditions

except for the difference of chromatographic matrix used. That is, CSP2 was prepared with silica I (5 μm, 200 Å, 200 m^2/g), while CSP4 was obtained with silica II (7 μm, 300 Å, 100 m^2/g). As is well known, the greater the pore size, the smaller the number of silanol groups on the silica surface. Therefore, the fixation of cellulose derivatives depends on the porosity of silica matrix. The characterization of CSP2 and CSP4 is reported in Table 2. It was found that in spite of the decrease in the number of silanol groups on the matrix, the percentage of the bonded chiral selector was improved instead. It seems possible that a part of silanol groups could be in the inner surface of silica I, where they might not be accessible to the TEPI-modified cellulose. Therefore, not all the silanol groups will be involved in the chemical immobilization in this case. While for the silica II, even if the quantity of silanol groups is lower, they can be readily accessible to the TEPI-modified cellulose because of the greater pore size. This may be a reason that the surface coverage of chiral selector on CSP4 was higher than that on CSP2.

The results for chromatographic enantioseparations are listed in Table 3. As can be shown, CSP4 generally exhibited higher chiral recognition abilities than CSP2 for the test solutes. In addition, the magnitude of α values of some enantiomers on CSP4 ever higher than those on CSP3 prepared with the regioselective fixation. For example, as seen in Fig. 5, the α value of warfarin on CSP4 (IV) was higher than those on the other phases (I to III) under the same chromatographic conditions. Resolution of drug candidate B was another representative example, as described in Fig. 8, a higher α value was seen on CSP4 (I) compared to that on CSP2 (II) under the same chromatographic conditions. Minguillón et al. [13] have investigated the influence of the porosity of the silica matrix on the bonding amount of cellulose derivative and performance of CSPs. According to their reports, the performance of the chiral stationary phases increased with pore size when the pore diameter was large enough to allow the penetration of macromolecules. Our present investigations are consistent with their results.

3.5. Separation of enantiomers on CSP5 and CSP6

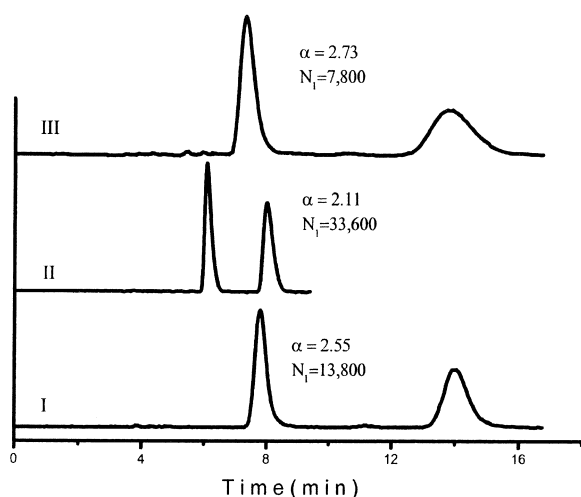In order to broaden the application range of the

Fig. 8. Chromatographic resolution of drug candidate B on CSP2 (II), CSP4 (I) and CSP6(III). Chromatographic conditions are described in Tables 3 and 4.

bifunctional reagent method developed here, two other types of CSPs based on cellulose 4-methylbenzoate (CMB, CSP5) and cellulose 3,5-dimethylphenylcarbamate (CDMPC, CSP6) were also prepared, respectively. As described in Table 2, CSP5 was prepared with the regioselective procedures, while CSP6 was obtained with the non-regioselective procedures. Their chiral resolving abilities were also evaluated by HPLC resolution of enantiomers. Both of them exhibited a good recognition capability for most of the test enantiomers from the chromatographic data of enantioseparations as listed in Table 4. For CSP5, the test enantiomers of warfarin (I) and Toger's base (II) were separated very well, as shown in Fig. 9, their respective α values were 2.56 and 2.11, respectively. As to CSP6, most of the test solutes were separated with baseline. A characteristic chromatogram is shown in Fig. 8, the α value for separation of drug candidate B was 2.71 (III).

In addition, the chiral recognition ability of CSP6 was compared to that reported by Yashima et al., in which they immobilized the same chiral selector to macroporous silica (1000 Å) with diisocyanate as the spacer reagent [10]. Their reported α values of enantiomers trans-stilbene oxide, benzoin and Troger's base on the non-regioselectively prepared CSP (2c-3) were 1.00, 1.16 and 1.00, and on the regioselectively prepared CSP (2a-3) were 1.30, 1.31 and 1.40, respectively, under the mobile phase of

Table 4
Chromatographic data for resolution of enantiomers on CSP5 and CSP6

Racemate	Mobile phase	CSP5		CSP6	
		k'_1	α	k'_1	α
trans-Stilbene oxide	a	0.42	1.26	0.44	1.15
Benzoin	a	1.37	1.14	1.68	1.25
Troger's base	a	0.52	2.11	0.84	1.52
Warfarin	b	0.71	2.56	0.80	1.50
Praziquantel	b	0.73	1.04	2.00	1.41
Bendroflumethiazide	b	0.53	1.00	1.52	1.14
Drug candidate A	c	3.56	1.02	2.52	1.21
Drug candidate B	c	3.04	1.12	1.03	2.73
α-DDBD	c	1.41	1.05	1.74	1.16
Ranolazine	d	1.25	1.00	1.59	1.75
3-Butyl-phthalide	e	0.65	1.00	1.27	1.06
Alprenolol	f	0.44	1.00	0.95	1.10
Metoprolol	f	0.80	1.00	1.59	1.16
Propranolol	f	0.72	1.00	1.38	1.38

Mobile phases: (a) hexane–2-propanol (90:10), (b) hexane–ethanol–THF–acetic acid (80:20:4:0.2), (c) hexane–ethanol–acetic acid (70:30:0.2), (d) hexane–ethanol–DEA (70:30:0.2), (e) hexane–ethanol (98:2), (f) hexane–ethanol–hexanic acid (70:30:0.2).

hexane–2-propanol (90:10). The corresponding α values on CSP6 prepared with the non-regioselective procedures were 1.15, 1.25 and 1.52 under the same mobile phase condition, as described in Table 4. It can be seen that the α values obtained here were higher to some degree than those reported by Yashima et al. on the non-regioselectively prepared
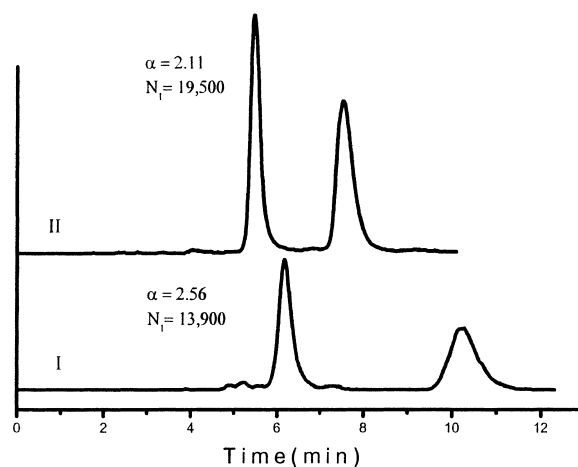

Fig. 9. Chromatographic resolution of warfarin (I) and Troger's base (II) on CSP5. Chromatographic conditions are described in Table 4.

phase (2c-3), but the α values of *trans*-stilbene oxide and benzoin were lower than their reported data obtained from the regioselectively prepared CSP (2a-3).

On the other side, it was clear seen that the chiral resolving power of 2c-3 was significantly poor than that of 2a-3 according to Yashima et al.'s reports [10]. That is to say, the regioselective procedures exhibited greatly more effectiveness than the non-regioseletive way with diisocyanates as the spacer reagent. However, no markedly difference in chiral recognition was observed between the two kinds of CSPs (CSP2 and CSP3) prepared with TEPI as the spacer reagent by non-regioselective and regioselective procedures, respectively. This might because when the diisocyanate was used as the spacer reagent, the cross-linking of cellulose derivative might be possibly occurred, especially for the non-regioselective procedures. But for TEPI, the cross-linking is greatly avoided since it contains two different functional groups. Therefore, the spacer of TEPI shows somewhat advantage than the spacer of diisocyanate for the non-regioselective preparation. These results may indicate that a bifunctional reagent containing two different functional groups has a potential to meet the requirement for a highly efficient synthesis of cellulose derivative-based CSPs.

Finally, it should be pointed out that the differences of the base materials used, such as chromatographic matrix, microcrystalline cellulose and steric structures of spacer reagents, might also affect the chromatographic separations.

4. Conclusion

It has been demonstrated that three types of CSPs based on cellulose phenylcarbamate, cellulose 4-methylbenzoate and cellulose 3,5-dimethylphenyl-carbamate were successfully prepared with the bifunctional reagent of 3-(triethoxysilyl)propyl isocyanate for effective enantioseparations. Enantioselectivities on the non-regioselectively prepared CSPs increased with the increment of the spacer reagent used for the synthesis. The regioselectively prepared CSP generally showed a higher resolution power than the non-regioselectively prepared CSP for the test enantiomers, while the latter phase showed great advantage from the view of rapid preparation. The CSPs based on the silica gel with pore size of 300 Å exhibited higher enantioselectivities than those prepared with pore size of 200 Å.

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