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Facile preparative HPLC enantioseparation of racemic drugs using chiral stationary phases based on mono-6^A-azido-6^A-deoxy-perphenylcarbamoylated β-cyclodextrin immobilized on silica gel

Siu-Choon Ng,[a,*] Lei Chen,[a] Li-Feng Zhang[b] and Chi-Bun Ching[c]

[a]*Department of Chemistry, National University of Singapore, Kent Ridge Crescent, Singapore 119260*
[b]*Environmental Technology Institute, Innovation Center Unit 237, Nanyang Drive, Singapore 639798*
[c]*Chemical and Process Engineering Center, National University of Singapore, Engineering Drive 4, Singapore 117576*

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Abstract—Immobilized chiral stationary phases (CSP)s from mono-6^A-azido-6^A-deoxy-perphenylcarbamoylated β-cyclodextrin were prepared using an extended application of the Staudinger reaction. Their application in preparative enantioseparations of racemic mixtures was demonstrated using atropine, bendroflumethiazide and four β-adrenergic blocking agents under reversed phase conditions. © 2002 Published by Elsevier Science Ltd.

Chiral compounds have recently aroused tremendous interest in many fields related to pharmaceuticals, natural products and agrochemicals. Moreover, detailed studies of the pharmacokinetics, physiological, toxicological, and metabolic activities of enantiomers of drugs have to be conducted since living systems comprise chiral proteins, nucleic acids and polysaccharides, with the consequence that living organisms may depict different physiological behaviors depending on the chirality of the drug enantiomers.[1] Consequently, analysis and purification of racemic drugs are of increasing importance and have attracted much attention over the past decade.

Asymmetric synthesis and optical resolution are two methods for obtaining enantiopure compounds. Of these, enantioresolution by high-performance liquid chromatography (HPLC) has been well explored in the past decade as one of the most direct and efficient methods for the determination of enantiomeric purity.[2,3] Meanwhile, crucial to this separation technique, several chiral stationary phases (CSPs) were prepared and commercialized for both analytical and preparative enantioseparations.[4]

Currently there are two main types of commercial CSPs. The first is prepared by physically coating functionalised polysaccharides, such as cellulose and amylose derivatives,[3c] onto silica gel and the latter is based on the chemical bonding of cyclodextrins (CD)[5] onto the silica gel by covalent bonds. These two types of CSPs exhibit good chiral separation abilities towards a broad range of structurally diverse racemic drugs and compounds. Although the mechanism of enantioseparation by these CSPs has not been realized completely, it is believed to be based on the interaction of the CSPs with the racemate to form transient diastereoisomers with different stabilities. As a result, pure enantiomers are eluted from the CSP with different retention times in a certain order.

The isolation of pure isomers is also a main target and the final goal in the development of CSPs. Although in the pharmaceutical industry there is still a strong dependence on classical fractional crystallization and application of achiral LC columns aided by chiral modifiers, CSPs have several advantages over the above two techniques. They are easily manipulated through synthesis and separate enantiomeric mixtures without the necessity of derivatization. Consequently, the preparative separation of chiral drugs and intermediates on CSPs is increasingly becoming a viable alternative to enantioselective synthetic routes, mainly ascribable to the advantages pertaining to time conservation and reliability of the scale-up procedure.

Keywords: β-cyclodextrin; enantioseparation; HPLC; preparative.
* Corresponding author. E-mail: chmngsc@leonis.nus.edu.sg

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However, scale-up procedures have some constraints that have to be circumvented. It is obvious that if an analytical method has been developed and if it can be applied for preparative scale, considerable amounts of time and cost will be saved. However, the reproducibility of procedures using CSPs, no matter whether for analytical or preparative scale, is another crucial consideration to the scale-up process.

In our previous work, we reported a novel and facile synthesis of a series of structurally well-defined CD-based CSPs by immobilization of monoazido-perfunctionalised β-CD onto aminized silica gel via a single stable urea linkage using the Staudinger reaction.[6,7]

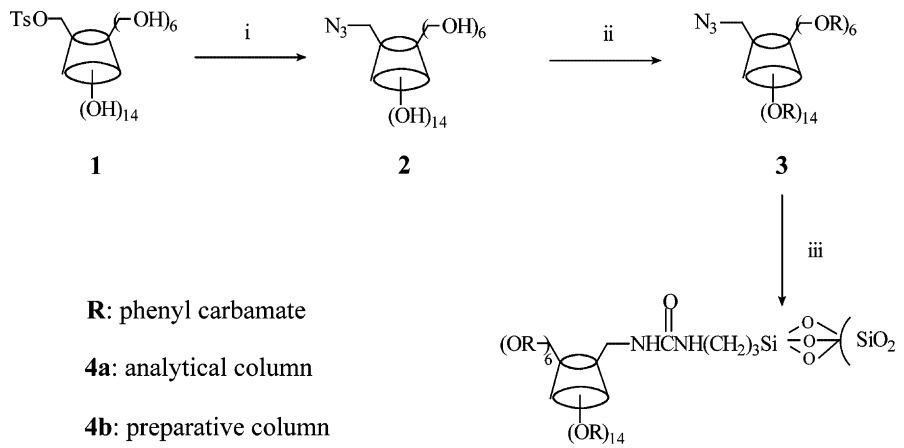
We report herein the scale-up and viability of applying the resulting CSP for preparative HPLC enantioseparations. It was found that this column possessed excellent enantioseparation ability towards several drugs under similiar reverse phase conditions to the analytical column.

With reference to Scheme 1, using the key intermediate mono-6^A-azide-6^A-deoxy-perphenylcarbamoylated β-cyclodextrin, **3**, reported previously,[7] two CSPs **4a** and **4b** were derived by immobilization onto two different types of aminized particulates which were Hypersil silica gel (particle size: 5 μm, pore size: 120 Å, surface area: 170 $m^2\ g^{-1}$) and Kieselgel 100 (particle size: 15–35 μm, pore size 100 Å, surface area 400 $m^2\ g^{-1}$). The derived CSPs were purified and thoroughly characterised.[8] Immobilization was evident from the weak but characteristic FT-IR vibrational bands in the 1800–1400 cm^{-1} region, reminiscent of those present in the precursor compound **3**. The higher carbon content in elemental analyses for **4a** and **4b**[9] and the surface concentration[10] of CD derivatives on the silica gel, ca. 0.32 and 0.06 μmol m^{-2}, further corroborated the success of our immobilization approach.

The CSPs were slurried in acetone and packed into standard stainless steel columns (analytical column: ϕ 4.6 mm×25 cm and preparative column: ϕ 10.0 mm×25 cm) on an Alltech® HPLC packer (Alltech Associates, Inc., USA) using acetone as solvent. The two sets of columns gave efficiencies of 28,000 and 10,000 plates per meter, respectively, using biphenyl as a test probe under normal phase (hexane and IPA in 90/10 v/v ratio).

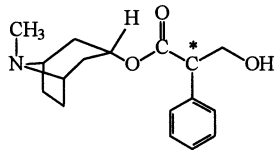
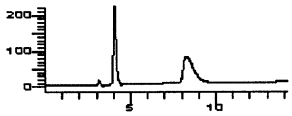
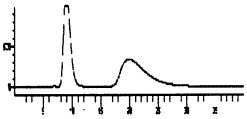
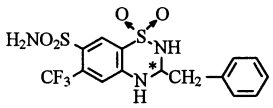
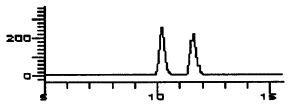
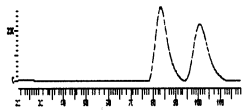
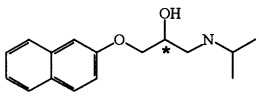
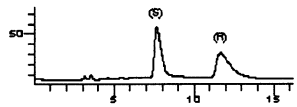
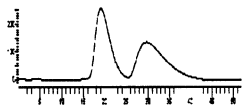
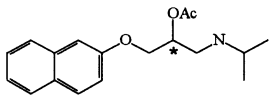
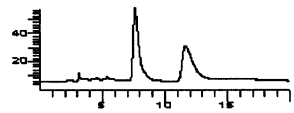
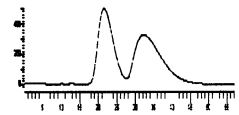
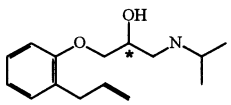
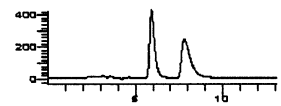
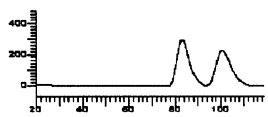
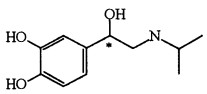
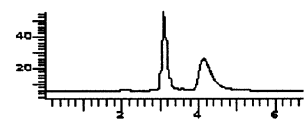
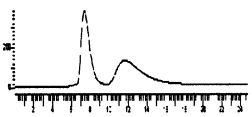
Analytical and preparative enantioseparation studies of propanolol, atropine, alprenolol, iso-proterenol, bendroflumethiazide and *O*-acetyl propranolol were accomplished under reversed phase with the chromatographic data obtained summarized in Table 1. From these results, it can be seen that most of the samples achieved excellent enantioseparation using mixtures of methanol in a buffer of 1% aqueous triethylammonium acetate as the mobile phases. However, for bendroflumethiazide, acetonitrile proved to be more effective on account of its stronger elution ability compared with methanol. Although the efficiency of the preparative column was lower than that of the analytical column because of the larger particle size of the silica gel with lower surface concentration of the chiral selectors, the optimal conditions for the enantioseparation of the drugs were similar in both cases. Among these drugs, atropine depicted the highest resolution (Rs) of 5.40 and 2.06 on the analytical and preparative columns, respectively. On the preparative column, iso-proterenol afforded the shortest retention times within 12 min with baselined-resolution, which paved the way for a facile preparative collection of isomers from multiple injection and collection of samples.

Interestingly, the elution orders of the *R* and *S* components of β-blockers on these two columns follow a similar sequence. Thus, with propranolol as a typical example, the elution order is always *S* before *R*. The similarity of both CSPs in selectivity toward the enantiomers indicated that the chiral recognition in the case of the small molecules is not influenced by the size and surface properties of the inert support, but by the polar carbamate groups, which can interact with the polar groups of the racemic compounds through hydrogen bonding and dipole–dipole interactions. Therefore, with



Scheme 1. Synthetic routes for two CSPs. *Reagents and conditions*: (i) NaN_3/DMF/95°C; (ii) phenyl isocyanate/pyridine/80°C/12 h; (iii) THF/aminized silica gel/CO_2/30°C/3 h.

Table 1. Chromatographic data for both analytical and preparative scale separation (mobile phases: A: 1% TEAA (pH 4.65), flow rate: 0.5 ml min^{-1}, detection: 225 nm. Conditions 1: A/MeOH = 65/35. Conditions 2: A/CH_3CN = 60/40. Conditions 3: A/CH_3CN = 70/30. Conditions 4: A/MeOH = 80/20)

	Racemes	Analytical column	Preparative column
1	Atropine 	 Conditions 1 k_1'=0.36, α=4.91, Rs=5.40	 Conditions 1 k_1'= 0.35, α=5.50, Rs= 2.06
2	Bendroflumethiazide 	 Conditions 2 k_1'= 2.40, α=1.21, Rs= 2.84	 Conditions 3 k_1'= 11.29, α=1.22, Rs= 1.51
3	Propranolol 	 Conditions 1 k_1'= 1.57, α=1.83, Rs= 3.26	 Conditions 1 k_1'= 1.80, α=1.86, Rs= 1.09
4	*O*-Acetyl Propranolol 	 Conditions 1 k_1'= 1.53, α=1.89, Rs= 3.24	 Conditions 1 k_1'= 3.73, α=1.70, Rs= 0.94
5	Alprenolol 	 Conditions 1 k_1'= 0.98, α=1.62, Rs= 2.46	 Conditions 4 k_1'= 2.90, α=1.63, Rs= 0.91
6	Isoproterenol 	 Conditions 1 k_1'=0.03, α= 11.6, Rs= 2.29	 Conditions 4 k_1'= 0.08, α=8.65, Rs= 1.20

the same immobilization procedure, the enantioseparation modes of the CSPs are similar.

The loading capacity is a critical factor in preparative separation. It indicates the maximum amount of racemate the column can tolerate without compromising resolution. In this paper, the loading capacity has been tested to demonstrate the potential of the preparative column for bulk scale separation. Therefore, the drugs chosen for this purpose should have good resolution and convenient capacity ratio (k') for the two enantiomers. The preparative separations performed under reversed phase conditions are listed in Table 1. Atropine was chosen as representative in depicting the procedure and principle in measuring the loading capacity, which is determined by k', α and Rs. Graphi-

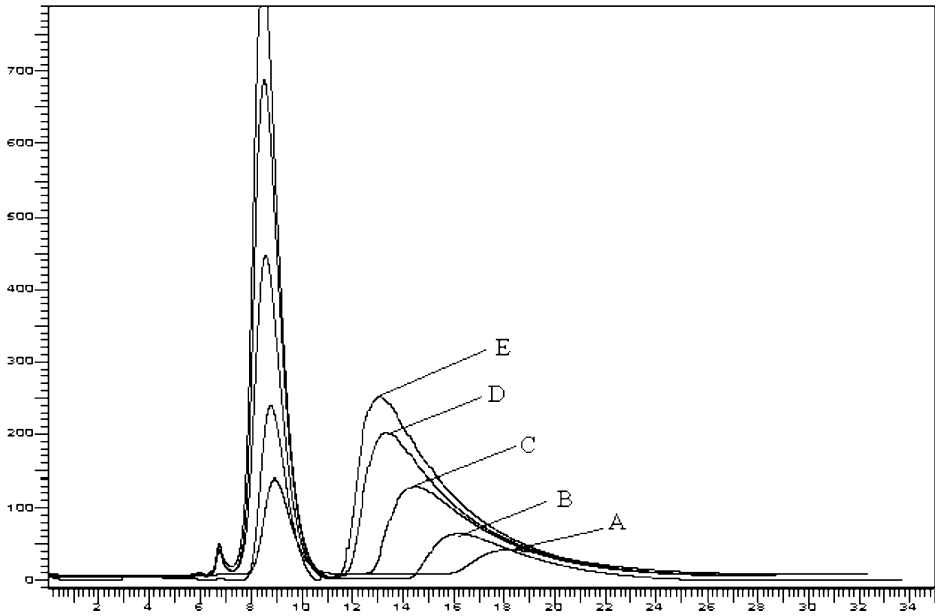


Figure 1. Chromatograms of atropine using the different injection amounts on SINUPHCD-Prep. (Mobile phase: 1% TEAA (pH 4.65)/MeOH = 65/35, flow rate: 2.0 ml min^{-1}, detection: 225 nm, injection volume: 50 μl. A: 43.6 mg ml^{-1}; B: 80.3 mg ml^{-1}; C: 160.1 mg ml^{-1}; D: 240.0 mg ml^{-1}; E: 250.0 mg ml^{-1}.)

cal representations of the influence of loading on k', α and Rs are shown in Figs. 1 and 2.

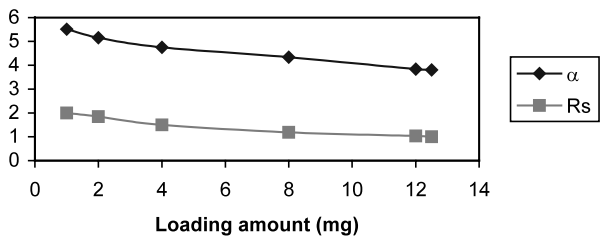
Fig. 1 depicts the chromatograms of atropine using different injection quantities. From A to E, the injection quantities are 2.18, 4.02, 8.0, 12.0 and 12.5 mg, respectively. From the chromatograms we observe that with an increase in loading of the sample, the retention of the two components decreases. From A to E, the two components eluted faster and the two peaks became closer. From the data we also observe the same trend in Fig. 2. In Fig. 2(b), k'_1, k'_2 are 0.35 and 1.93, respectively, when the injection amount was 2.0 mg. They decreased with increasing injection amounts and reached lowest values of 0.25 and 0.96, respectively, when the loading was 12.0 mg. Even when the loading is increased to 12.5 mg on a preparative column, atropine can still achieve baseline resolution. However, the decrease of k'_2 is larger than that of k'_1. As a result, k'_1 and k'_2 were down to 70 and 50%, respectively, of the initial value.

From Fig. 2(a), it is evident that α and Rs decrease from A to E. When the amount injected is 1.0 mg per injection, α is 5.51 and Rs is 2.0. However, they drop to 5.15 and 1.85, respectively, when the injection amount is 2.0 mg. With the loading increased from 4.0 to 12.0 mg, α and Rs decreased from 4.75 to 3.84 and 1.5 to 1.03. Therefore, the loading capacity of atropine on a preparative column was determined as 12.5 mg when the Rs value reached 0.90.

Using similar approaches, the loading capacities of other drugs were determined as 1.06, 1.11, 1.02 and 1.06 mg for bendroflumethiazide, propranolol, alprenolol and isoproterenol, respectively. Amongst the analytes, *O*-acetyl propranolol had the lowest loading of 0.94 mg.

The optical rotations of propranolol,[11] isoproterenol[12] and atropine were determined and are summarized in Table 2. The recovery which is important in preparative chromatography was generally high, above 90%.

(a) Effect of loading amount on α and Rs



(b) Effect of loading amount on k′

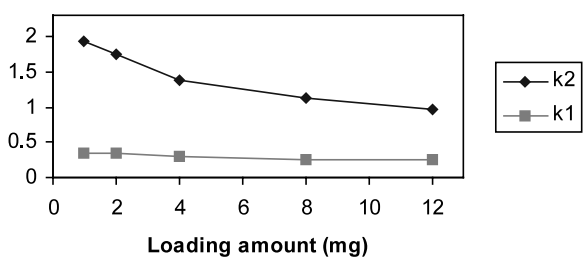


Figure 2. Effects of the loading amount on k', α and Rs of atropine on SINUPHCD-Prep. (Mobile phase: 1% TEAA (pH 4.65)/MeOH = 65/35, flow rate: 2.0 ml min^{-1}, detection: 225 nm, injection volume: 50 μl.)

Table 2. Optical rotation of propranolol, isoproterenol and atropine. (Solvent: EtOH, temperature: 20°C for propranolol and isoproterenol while 25°C for atropine)

Drugs	α	Literature value	Yield (%)
Isoproterenol	$\alpha_1 = +49.6$ ($c=1$, EtOH)	Literature: ± 50	99.2
	$\alpha_2 = -49.8$ ($c=1$, EtOH)		99.6
Propranolol	$\alpha_1 = +30.8$ ($c=1$, EtOH)	Literature: ± 32	96.2
	$\alpha_2 = -31.2$ ($c=1$, EtOH)		97.5
Atropine	$\alpha_1 = +24.3$ ($c=1$, EtOH)	NA	
	$\alpha_2 = -22.4$ ($c=1$, EtOH)		

On the basis of the experimental results presented above, preparative column chromatography on our CSP proved effective for the isolation and collection of pure drug isomers. Enantiomers of β-blockers, atropine and bendroflumethiazide were successfully resolved on these two CSPs. The baseline of the chromatograms appeared stable and the results obtained are readily reproducible. The preparative column also exhibited good stability under a wide range of conditions and is efficient for preparative enantioseparation and collection of pure isomers.

Acknowledgements

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8. For **4**: Light yellow powder, IR (cm^{-1}): 3401, 3308 (N–H str), 2102 (N_3 str), 1738 (C=O str), 1549, 1505 (arom C=C ring str), 1215, 1043 (C–O–C str), 772 (C–H atom op bend).
9. Elemental analysis: **4a** C, 17.42; H, 2.15; N, 2.56% and for **4b**, C, 7.54; H, 1.07; N, 1.22%.
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11. $[\alpha]_D^{20}$ for propranolol is: the first peak: +49.6 ($c=1$, ethanol), second peak: −49.8. (Literature value: ±50, Merck Catalogue, 1999/2000.)
12. $[\alpha]_D^{20}$ for isoproterenol is: the first peak: +30.8 ($c=1$, ethanol), second peak: −31.2. (Literature value: ±32.0, Merck Catalogue, 1999/2000.)