Cellulose Derivative-based Beads as Chiral Stationary Phase for HPLC

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Crosslinked beads, which can be used as a chiral packing material (CPM) for high-performance liquid chromatography (HPLC), were prepared using a cellulose phenylcarbamate derivative. The spherical beads were first prepared with partially derivatized cellulose 3,5-dimethylphenylcarbamate, which was then crosslinked with a diisocyanate. The obtained beads had a higher loading capacity than the corresponding conventional CPM prepared by coating the derivative on silica gel, and were not damaged when used with an eluent containing chloroform which cannot be used with the coated CPM.

During the past two decades, a large number of chiral stationary phases (CSPs) for HPLC have been prepared, and nearly 100 CSPs are now commercially available.[1] Among the many CSPs, the phenylcarbamate derivatives of cellulose and amylose appear to be the most frequently used for a wide range of racemates. These types of CPMs have been prepared by coating polysaccharide derivatives by ca. 20 wt % on macroporous silica gel as a support.[1b] However, the amount of the derivatives, which can be coated on silica gel, is limited; therefore, the coated-type CPMs may not be the best ones for preparative separation. To overcome this defect, the beads consisting of only polysaccharide derivatives seem to be favorable. However, such beads have not yet been successfully prepared with the phenylcarbamate derivatives of cellulose and amylose, although cellulose benzoate beads have already been prepared by Francotte et al.[2]

In this study, cellulose 3,5-dimethylphenylcarbamate (CDMPC) beads as a new CPM were prepared from CDMPC containing a small amount of hydroxy groups, which can be used for crosslinking with a diisocyanate. The obtained insoluble beads were packed into an HPLC column and their chiral recognition ability was evaluated.

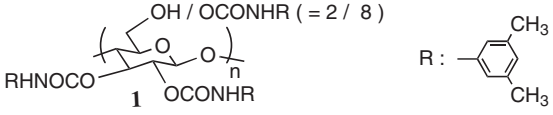

Figure 1. Structure of cellulose derivative **1**.

A cellulose derivative (**1**) having ca. 20% hydroxy groups at the 6-position was prepared according to a previous method (Figure 1).[3] First, the cellulose was swollen in *N*,*N*-dimethylacetamide containing LiCl at 80 °C for 24 h, and then pyridine and triphenylmethyl chloride were added to react at 80 °C. After 24 h, an excess of 3,5-dimethylphenyl isocyanate was added and allowed to react for 24 h. The obtained derivative was isolated as a methanol-insoluble fraction and suspended in methanol containing a small amount of hydrochloric acid in order to deprotect the triphenylmethyl group at room temperature. The obtained cellulose 2,3-bis(3,5-dimethylphenylcarbamate) was reacted with a calculated amount of 3,5-dimethylphenyl isocyanate in pyridine at 80 °C for 24 h in order to obtain **1**. The structure of **1** was confirmed by its ^{1}H NMR spectrum taken in pyridine-d_5 at 80 °C with a Varian Gemini-2000 400 MHz NMR instrument. A solution of **1** (0.25 g) dissolved in a THF–1-heptanol (2/1, v/v) mixture (30 mL) was dropwise added to water (500 mL) containing sodium lauryl sulfate (0.2%) with mechanical stirring at 1100 rpm in a 1 L flask at 75–80 °C.[2a] The stirring and temperature were maintained until the THF was almost completely removed by evaporation. The suspension was then filtered to separate the beads, which were first washed with water and then with methanol, and dried in vacuo at 60 °C for 12 h. The diameter and surface area of the beads were determined to be 5–8 μm by SEM (Figure 2) and 2.6 m^2/g by the BET method, respectively.[4] In order to increase the mechanical strength and enhance the durability of the beads against solvents, the residual hydroxy groups in the beads were crosslinked with 4,4′-diphenylmethane diisocyanate (1.5 equiv. to the residual hydroxy groups) in toluene at 80 °C for 12 h. The obtained beads were used as a packing material after being washed and dried. The crosslinking degree and the structure change of **1** are still unknown.[5]

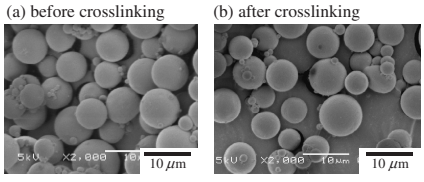

Figure 2. SEM images of the cellulose derivative beads.

When we tried to prepare beads using the completely carbamoylated CDMPC under the same conditions, the beads were not successfully formed. The existence of a small amount of unreacted hydroxy groups may be necessary to form the beads. For comparison, a coated-type CPM was prepared by coating pure CDMPC (20 wt % of silica gel) on the macroporous silica gel (SP-1000 Daiso gel, 7-μm diameter, 100-nm pore size).[1b] Each of the beads and the coated-type CPM were packed in a stainless-steel tube (25 × 0.20 cm (i.d.)) by a slurry method. The two columns contained approximately 0.27 and 0.06 g CDMPC, and had the plate numbers of 2300 and 1400, respectively, for benzene using a hexane–2-propanol (90/10, v/v) mixture as the eluent at a flow-rate of 0.1 mL/min. 1,3,5-Tri-*t*-butylbenzene was used as a non-retained compound to estimate the dead time (t_0).[6]

Figure 3 shows the chromatogram for the resolution of racemic cobalt(III) tris(acetylacetonate) (**9**) on the bead column. The enantiomers were eluted at retention times of t_1 and t_2 with complete separation. The dead time (t_0) was estimated to be

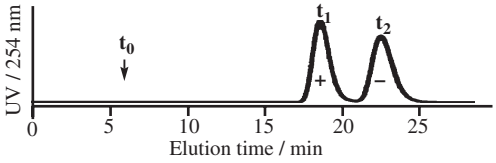

Figure 3. Chromatogram for the resolution of $Co(acac)_3$ **9** on the bead column.

6.76 min using a hexane–2-propanol (90/10, v/v) mixture as the eluent at a flow rate of 0.1 mL/min. The capacity factors, $k_1'[= (t_1 - t_0)/t_0]$ and $k_2'[= (t_2 - t_0)/t_0]$, were 1.71 and 2.28, respectively, which led to the separation factor α $(= k_2'/k_1')$ of 1.33. The results of the enantioseparation of ten racemates (**2**–**11** in Figure 4) on the bead column are summarized in Table 1, together with those of the coated-type column. Because the bead column consists of only the cellulose derivative without the silica support, it had much higher capacity factors than the coated-type column.

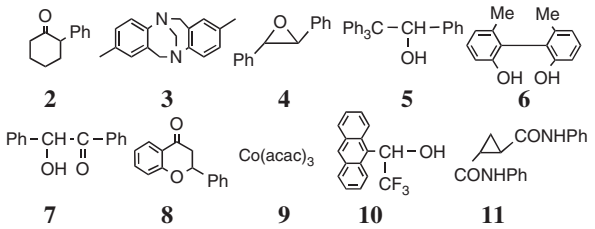

Figure 4. Structures of racemates **2**–**11**.

Table 1. Resolution of racemates (**2**–**11**) on the bead and the coated-type columns

Run	Bead		Coated-type	
	k_1'	α	k_1'	α
2	3.75 (−)	1.19	0.84 (−)	1.11
3	3.45 (+)	1.20	0.79 (+)	1.21
4	2.35 (−)	1.67	0.54 (−)	2.33
5	4.91 (+)	1.18	1.02 (+)	1.33
6	5.34 (−)	2.77	2.04 (−)	1.70
7	8.18 (+)	1.33	2.71 (+)	1.64
8	4.79 (−)	1.21	1.03 (−)	1.45
9	1.71 (+)	1.33	0.29 (+)	ca. 1
10	8.84 (−)	2.77	1.43 (−)	3.28
11	3.73 (+)	1.40	0.52 (+)	3.21

Column, 2.0 mm (i.d.) × 250 mm. Eluent, hexane–2-propanol (90:10, flow rate, 0.1 mL/min). The signs in parentheses represent the optical rotation of the first-eluted enantiomer.

The bead column shows a high chiral recognition ability similar to the coated-type column and the elution orders of the enantiomers on the two columns are the same, although the separation factors for some racemates on the two columns are somewhat different, which may be in part ascribed to the absence of the non-selective adsorption by the silica support. The CDMPC residues in the beads may have a slightly different higher order structure from the CDMPC on the coated-type CPM.[3,7]

The loading capacities for a racemate, 2,2,2-trifluoro-1-(9-anthryl)ethanol (**10**), on the two columns were compared (Figure 5). The amounts of the sample were changed between 0.01 and 20 mg. The bead column almost completely resolved 10 mg of the sample, while the coated-type column exhibited a slight overlap of peaks for the 6-mg injection. These results indicate that the bead column has a higher loading capacity than the coated-type column, at least for this compound.

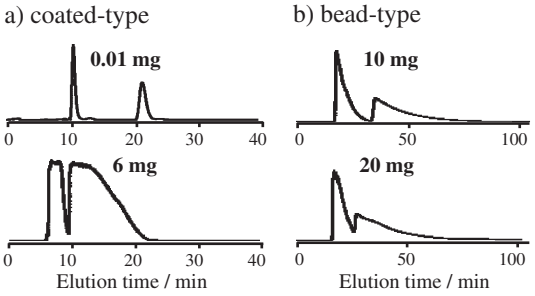

Figure 5. Resolution of 2,2,2-trifluoro-1-(9-anthryl)ethanol (**10**). Column, 2.0 mm (i.d.) × 250 mm. Eluent, hexane–2-propanol (90:10). Flow rate, a: 0.20 mL, b: 0.15 mL/min.

Solvents such as chloroform and tetrahydrofuran cannot be used as the eluent for the coated-type CSPs, because these solvents dissolve or swell the polysaccharide derivatives coated on the silica gel. However, the crosslinked beads prepared here do not prevent the use of these solvents,[8] because they are totally insoluble in the solvents. This is another merit of the beads. The merits of immobilized CSPs have already been discussed.[8] The selection of a suitable eluent is very important not only for analytical separations but also for preparative ones.

In summary, the crosslinked beads of CDMPC were successfully prepared and used as the CPM for HPLC. The beads showed a high chiral recognition ability comparable to that of the conventional coated-type CPM and possessed a higher loading capacity, which is particularly important for preparative separations. The crosslinked beads can be used with chloroform as the eluent.

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4 If the beads size can be uniformed, the plate number may become higher and the more effective resolution may be achieved.
5 When the crosslinked beads were further treated with *tert*-butanol in order to convert the residual isocyanate groups to carbamate residues, the separation factors for several compounds are increased.
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