Preparation of Three Different Style Packed Capillary Frits

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Received December 23, 2005

Three different style capillary columns, a packed capillary with temporary quartz wool frit, a packed capillary with immobilized frit, and an immobilized packed-capillary, were easily prepared with a commercially available (S)-N-(3,5-dinitro-benzoyl)leucine-N-phenyl-N-alkylamide derived chiral stationary phase. Liquid chromatographic chiral separations of some racemic amino acid derivatives on these columns were performed and the results were compared to each other. The packed capillary with immobilized frit showed some merits in chiral chromatography.

Key Words : Different style frit, Packed-capillary, Immobilized frit, Temporary frit, Chiral chromatography

Introduction

Capillary HPLC is a very useful and attractive technique in enantioseparation since it is easily applicable to test a newly developed chiral stationary phase with very small amount of packing material. Additionally, it can be applied to a newly commercialized chiral stationary phase that is normally very expensive. In addition, it can reduce the consumption of expensive chiral mobile phase additives, chiral samples,[1] and the consumption and disposal of HPLC solvents.[1-6] There are three different types of capillary columns: a packed capillary column,[7,8] a coated capillary column,[9] and a monolithic capillary column.[10,11] Among them, the packed capillary is more practical then others because it can be easily applied to large scale separation. Various methods have been suggested for packing silica particles into capillaries to make capillary columns.[12-14] Three methods related to the production of packed capillary frits, sintering silica gel,[15] sol-gel method,[16] and photopolymerization,[17] were reported and compared to each other.[18]

In the current study, we tried to find an easy and very simple method to prepare capillary frit without using the sintering or polymerization method. First of all, three different style capillaries, a packed capillary with temporary quartz wool frit, a packed capillary which has sodium silicate immobilized frit, and an immobilized capillary made with sodium silicate solution, were prepared with the same stationary phase (CHIRALHYUN-LEU-1).[19] Next, liquid chromatographic chiral separations of various racemic amino acid derivatives on these columns were performed and the results were compared with each other.

Experimental Section

Apparatus. A DSF-122 Air Driven Fluid Pump (Haskel, USA) was used for slurry packing of a conventional column (4.6 mm ID, 25 cm length). A Model PU-2080 Plus Intelligent pump (JASCO, Tokyo, Japan) was used for slurry packing of the capillary column (0.25 mm ID, 20 cm length). A capillary column washer was purchased from Alltech Korea (Seoul, Korea). An HPLC system constructed with a JASCO (Tokyo, Japan) Model PU-2080 Plus Intelligent pump, a Rheodyne (Cotati, CA, USA) Model 7125 injector with 20 μL sample loop, a JASCO (Tokyo, Japan) Model UV-2075 Plus Intelligent UV/Vis detector, and a JASCO (Tokyo, Japan) Model OR-2090 Plus Chiral detector were used for evaluation of the conventional column. A capillary HPLC system with a Valco Micro Injector, Model INJ-P4-100 (sample volume: 100 nL), Knauer Wellchrom Variable UV-VIS Detector K-2501, Model No. A4180 (capillary cell, cell volume 35 nL) were used for evaluation of the capillary columns. SEM data were obtained from JSM-5400 Scanning Electron Microscopy (Jeol, Japan).

Materials and Chemicals. Methanol, acetone, 2-propanol, and n-hexane were HPLC grade and obtained from Merck Korea (Seoul, Korea). The chiral stationary phase (CSP 1), CHIRALHYUN-LEU-1 (Figure 1), was a 5 μm and spherical shape Pirkle type CSP obtained from K-MAC (Daejeon, Korea).[20] Fused silica capillaries (0.53 mm ID, 30 m length; 0.25 mm ID, 60 m length) were purchased from Alltech Korea (Seoul, Korea) and liquid sodium silicate was purchased from Samjeon chemical (Seoul, Korea). The sodium silicate mixed solution was prepared by mixing the

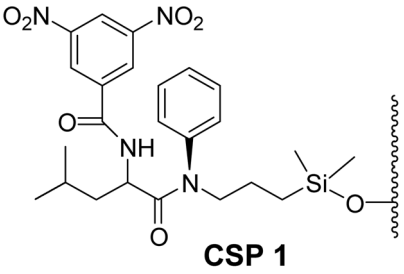


Figure 1. Structure of chiral stationary phase (CSP 1).

liquid sodium silicate and methanol in 7 : 3 ratio. The test racemic compounds were synthesized products of previous work (Figure 2).[19] Various tubes (polyethylene, Teflon, and stainless steel) used for making capillaries were purchased from many Japan companies.[21]

Preparation of Capillary Columns (Figure 3)

(1) An end of 10 mm length, 0.21 mm ID, and 0.80 mm OD polyethylene tube was heated and put onto the 0.25 mm ID, 0.65 mm OD, and 20 cm (or 10 cm) length capillary.

(2) An 8 mm length, 0.30 mm ID, and 0.80 mm OD Teflon tube was heated and put onto the polyethylene covered capillary.

(3) A 0.25 mm ID, 0.35 mm OD, and 10 mm length stainless tube was put into the end of 0.21 mm ID polyethylene tube, and then the end of 10 mm length, 0.21 mm ID, and 0.80 mm OD polyethylene tube was heated and put onto the stainless tube.

(4) A thin thread of quartz wool was introduced into the capillary polyethylene tube area. The quartz wool was tightly packed by pushing with stainless steel rod.

(5) An end of 10 mm length, 0.21 mm ID, and 0.80 mm OD polyethylene tube was heated and put onto the other end of the capillary. The capillary was washed consecutively with water, 0.1 M NaOH, water, and acetone, each time for about 5 min at a pressure of 5 bar by using capillary column washer. Meanwhile, 100 mg of CSP 1 was slurried in 5 mL acetone, and ultrasonicated for 10 min. The slurry was rapidly introduced into the 5 cm length, 4.6 mm ID empty column. The slurry containing column was immediately connected to the pump and the washed capillary. The pump was switched on and the flow rate was increased slowly to 0.10 mL/min. As soon as the pressure reached more than100 bar, the flow rate was reduced to and maintained around 100 bars. The pump was switched off when the capillary was filled with the stationary. (The packing line was visible to the naked eyes.)

(6) At the open end of the capillary, a thin thread of quartz wool was introduced into the capillary stainless steel area and was tightly packed again (Packed capillary Type-I; Packed capillary with temporary quartz wool frit; Takeuchi method).

(7) A sodium silicate mixed solution was introduced to the both end area of the Type-I packed capillary using a syringe.

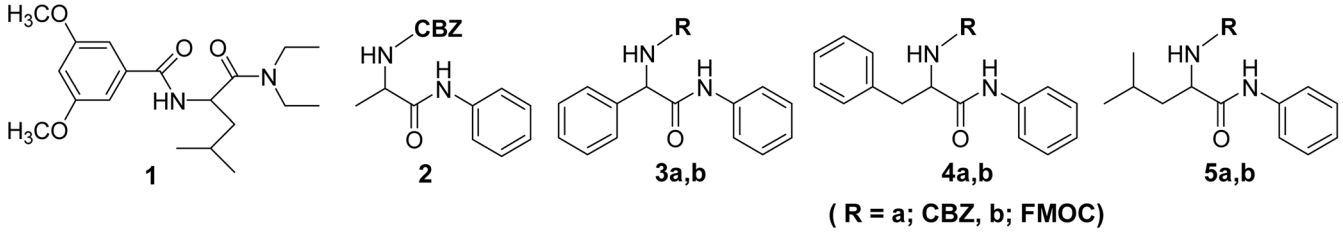


Figure 2. Structure of amino acid derived chiral analytes. (CBZ: carbobenzyloxy, FMOC: 9-fluorenylmethoxycarbonyl).

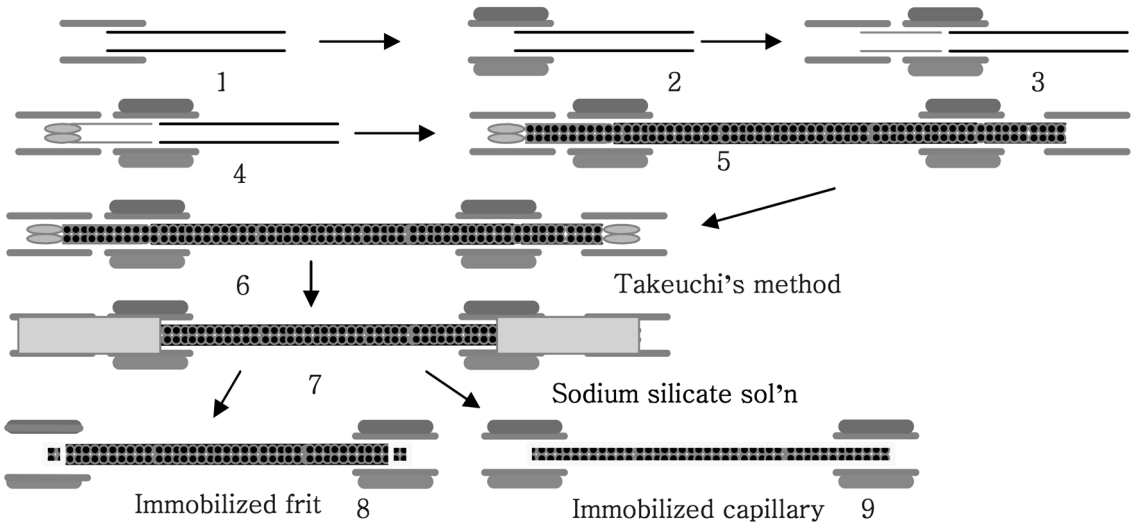


Figure 3. Procedure for the preparation of chiral capillary columns.

The capillary was dried at 90 °C for 20 min.

(8) The 0.25 mm ID stainless tube and 0.21 mm ID polyethylene tube connected in the process (3) was removed from the capillary (Packed capillary Type-II; Packed capillary with immobilized frit).

(9) A sodium silicate mixed solution was introduced into all areas of the Type-I packed capillary and dried at 90 °C for 20 min. A 0.25 mm ID stainless tube and 0.21 mm ID polyethylene tube connected in the process (3) was removed from the capillary (Packed capillary Type-III; Immobilized packed-capillary).

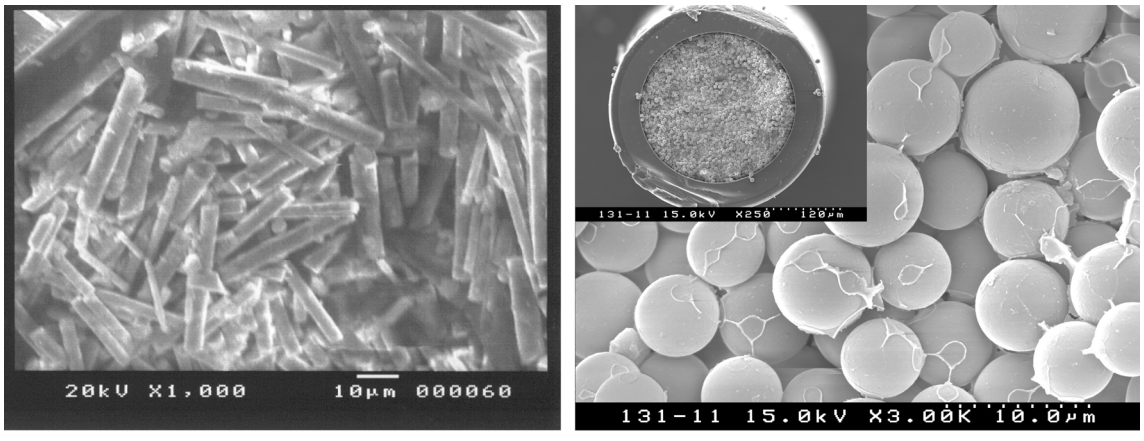
Chromatography (Capillary Testing). 20% IPA in hexane (flow rate: 15 μL/min or 1.5 mL/min) was used as the mobile phase and **CSP 1** was used for the packing material (See Figure 1). The racemic amino acids derivatives, **1-5a, b** were used for test samples (See Figure 2). The column void volume was checked by injecting 1,3,5-tri-*tert*-butylbenzene.[22]

Results and Discussion

Two SEM images of the capillary column frit region prepared by two different methods are shown in Figure 4. In Figure 4(a), about 50-micron length quartz wool rods having 5-7 micron wide are shown. The quartz wool rods were prepared by breaking the quartz wool threads in the course of pushing them in preparation step (4) of the capillary columns. Because of this, the quartz wool rods were arranged irregularly and could breed the 5-micron stationary phases with a small shock or pressure change. A picture of the capillary column frit region immobilized by a sodium silicate mixed solution is shown in Figure 4(b). As shown in magnified picture, the hardening sodium silicate connected each silica gel particle as glue and immobilized the stationary phase. A SEM image of an immobilized capillary made with sodium silicate solution (Type II) was very similar with that of Type III capillary. D,L-leucine derived compound **1** was used to test the efficiency of the capillaries prepared on four kinds of columns including the conventional column, and the resolution results are shown in Figure 5. Excellent results were obtained on the conventional column, while the worst results were obtained on the Type-III packed capillary. The selectivity factor (α), resolution (Rs), and number of theoretical plate (N) calculated from these chromatograms are shown in Table 1.

The N value of the conventional column (Figure 5a) was 4,100 (16,400 N/m), the largest value, those of Type-I and Type-II capillaries were 2,000-4,000 N/m, while that of Type-III capillary was 1,000 N/m, the smallest value. Because we did not optimize the packing condition until now, the theoretical number is worse than previous report.[12-17] Because both ends of the stationary phase in Type-II capillaries were encroached upon by sodium silicate, it was expected that the N value of Type-II capillaries would be smaller than that of Type-I capillaries. However, some diffusion occurred in the temporary quartz wool frit region of Type-I capillaries and this diffusion decreased the N value and resulted in similar N values in both types of capillaries. In addition, this diffusion affected the small N value difference between the two different length Type-I capillaries. In Type-II capillaries, the actual length of the stationary phase excluding the encroached area in long (20 cm) capillaries is about 19 cm, while that in short (10 cm) capillaries is about 9 cm. A large difference was found in the two different length Type-II capillaries. Even though the number of theoretical plate (N) values between Type-I and Type-II is similar, the Type-II capillary has many merits in chromatography. The Type-II and Type-III capillaries can be made within two hours without any difficulty. Therefore, if someone wanted to make a test column with small amount of a stationary phase, one can easily make the test column without any special skill. The easy preparation of chiral capillaries allows for a high throughput screening of potential chiral selectors.

In addition, the stationary phases in the frit region are bonded to each other, the frit region in the Type-II and Type-III capillaries is stronger than that in other type capillary, especially sintered frit. An additional advantage in using the sodium silicate solution to form frit is physical and chemical



(a) (b)

Figure 4. SEM images of capillary column frit region; (a) A packed capillary with temporary quartz wool frit (Type I), (b) An immobilized capillary (Type II or Type III).

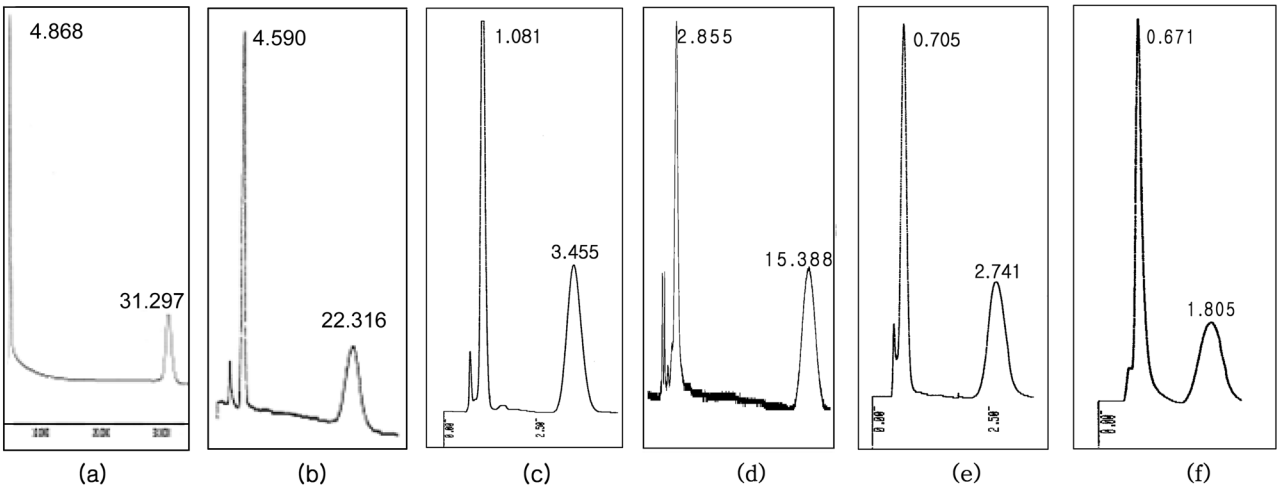


Figure 5. Chromatogram of the resolution of racemic **1** on (a) Conventional column, (b) A 20 cm length packed capillary with quartz wool temporary frit (Type I), (c) A 10 cm length packed capillary with quartz wool temporary frit (Type I), (d) A 20 cm length packed capillary which has sodium silicate immobilized frit (Type II), (e) A 10 cm length packed capillary which has sodium silicate immobilized frit (Type II), (f) An immobilized capillary made with sodium silicate solution (Type III). All chromatograms were obtained using 20% 2-propanol in hexane as mobile phase. For other conditions see the footnote of Table 1.

Table 1. Chromatographic factors for sample **1** on four different style columns

	Conventional column[b]	Capillary column Type I		Capillary column Type II		Capillary Type III[d]
		long[c]	short[d]	long[c]	short[d]	
α	11.93	8.12	6.58	8.30	8.71	6.53
Rs	20.0	6.0	4.6	8.0	4.2	2.4
N^e	4,100	420	360	600	250	100
N/m	16,400	2,100	3,600	3,000	2,500	1,000

[a]All data have been collected using 20% 2-propanol in hexane as a mobile phase at the flow rate 1.5 mL/min for conventional column and 15 μL/min for capillary columns. Void volumes were measured using TTBB(1,3,5-tri-tert-butylbenzene). [b]Column size; 4.6 mm ID, 25 cm length. [c]Column size; 0.25 mm ID, 20 cm length. [d]Column size; 0.25 mm ID, 10 cm length. [e]Number of theoretical plate.

stability. There is no exposure of UV light or high temperature on the frit region in the Type-II capillary and the sodium silicate solution does not react with the functional group of any stationary phase, Type-II capillary is physically stronger and chemically more stable than other type capillaries.

The chiral separation of sample **2-5b** was performed on the six columns. The capacity factor (k) and selectivity factor (α) are shown in Table 2. The best results were obtained on the conventional column and are similar with the *N* values shown in Table 1. As the amount of sodium silicate increased, the elution time was shortened. This phenomenon can be explained with Figure 4; as explained before, the sodium silicate served as a glue that covered the surface of the stationary phase and that interfered with the interaction between the stationary phase and the samples. Among the three different type capillaries in this study, the Type-II capillary shows better results than others. Therefore, the preparation method of the Type-II capillary can be used as one of fast and easy method for making packed capillary.

Table 2. Comparison of the enantioseparation results for **1-5b** on four different columns[a]

Analytes	Conventional column[b]		Capillary column Type I				Capillary column Type II				capillary Type III[d]	
			long[c]		Short[d]		long[c]		short[d]			
	k_1	α	k_1	α	k_1	α	k_1	α	k_1	α	k_1	α
1	1.83	11.93	1.19	8.12	0.71	6.58	1.51	8.30	0.60	8.71	0.45	6.53
2	0.85	5.04	0.76	4.53	0.72	2.91	1.09	4.74	0.52	4.00	0.43	2.71
3a	1.87	2.32	1.36	2.47	1.00	1.97	1.44	2.86	0.82	2.23	0.49	1.90
3b	2.17	2.57	1.58	2.68	1.19	2.11	1.63	3.08	0.96	2.42	0.55	2.18
4a	0.97	5.74	0.99	4.02	0.82	2.88	1.06	5.48	0.53	4.21	0.37	3.17
4b	1.04	6.01	0.91	3.27	0.80	3.13	0.97	5.37	0.60	4.24	0.37	3.60
5a	0.58	7.50	0.64	5.45	0.60	3.62	0.64	6.62	0.33	5.23	0.27	3.46
5b	0.66	7.52	0.76	6.86	0.67	3.66	0.58	6.78	0.39	5.24	0.28	3.38

[a]All data have been collected using 20% 2-propanol in hexane as a mobile phase at the flow rate 1.5 mL/min for conventional column and 15 μL/min for capillary columns. Void volumes were measured using TTBB(1,3,5-tri-tert-butylbenzene). [b]Column size; 4.6 mm ID, 25 cm length. [c]Column size; 0.25 mm ID, 20 cm length. [d]Column size; 0.25 mm ID, 10 cm length.

Acknowledgements. This work was supported by the Korea Research Foundation Grant (KRF-2003-00178).

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