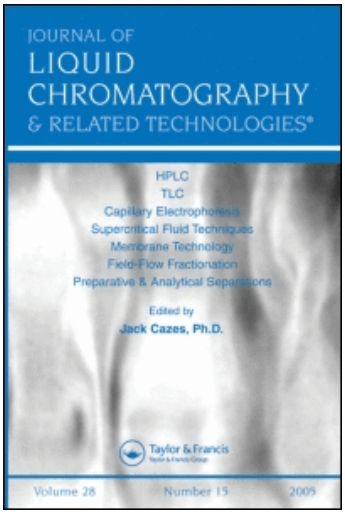

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Semimicro Size Exclusion Chromatography For Oligomers. I. Column Packing Procedure

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SEMIMICRO SIZE EXCLUSION CHROMATOGRAPHY FOR OLIGOMERS. I. COLUMN PACKING PROCEDURE

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ABSTRACT

Polystyrene gels of a particle diameter 10 ± 2 μm for the use in oligomer separation were packed into 1.5 mm i.d. x 25 cm length columns by the balanced density slurry-packing technique under a constant flow rate of 500 μL/min. The slurry solvent was a mixture of toluene and chloroform (50.5/49.5, v/v). The example of the number of theoretical plates (N) of these columns was 8600 plates/25 cm (HETP = 29.1 μm) at flow rate of 40 μL/min by injecting 1 μL of 0.5% benzene solution. Sixteen columns were connected and the overall value of N was 103000 plates/4 m. A typical example of oligomer separation was demonstrated. A constant-flow technique is preferable to a constant-pressure technique. When two or three column blanks were packed together, the columns located at the outlet of the packer-column assembly had higher values of N. Optimum flow rate of the slurry solvent when three column blanks were packed together lay between 400 and 500 μL/min. The packing efficiency, that is, the probability of getting valid columns was about 60%. Viscous slurry solvents were not effective to get efficient columns. To pack gels in the less swollen state gave sometimes efficient columns. Pressure monitoring in progress of packing was very effective to foresee the column efficiency.

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INTRODUCTION

Miniaturization of HPLC is useful for saving on solvents in addition to fabricating columns at reasonable costs in lengths. In our previous papers [1,2], we showed how to pack polystyrene (PS) gels, which are exclusively used for size exclusion chromatography (SEC), into 1.8 mm i.d. or 1.5 mm i.d. columns and to obtain high-quality columns. Operational variables such as the effects of mobile phase velocity and injection volume on the number of theoretical plates (N) and retention volumes were also discussed in addition to the comparison with conventional columns. However, the PS gels used in the experiment were those for the use in polymer separation, not for oligomers. The PS gels for the separation of oligomers are supposed to be less rigid than those for polymers and the column packing procedure might be different between them. In addition, higher number of theoretical plates is required to resolve oligomers into each degree of polymerization, because the selection of the mobile phase and the peak capacity are restricted in SEC. Making a longer column or connecting many columns in series at reasonable costs would be possible by semimicro SEC.

In this paper we discuss the procedure to pack PS gels for oligomers into a column of 1.5 mm i.d. and 25 cm length. In part II [3], sixteen columns will be connected to get higher values of N and several oligomers will be separated, the results being compared with conventional SEC (8 mm i.d. columns).

EXPERIMENTAL

PS gels used in this experiment were taken from a Shodex A802 HPSEC column, which have nomial exclusion limit of 8000 molecular weight, as polystyrene, and have a particle diameter 10 $\pm$ 2 μm. A packer was an empty column of 7.2 mm i.d. x 25 cm length, which was connected to the inlet of a chromatographic column blank of 1.5 mm i.d. through a reducing union. Column packing was performed by using a Jasco TRIROTAR-V pump which can deliver a solvent from 10

μL/min to 9.9 mL/min at a 10-μL interval in the semimicro mode and at 0.1 mL interval in the normal LC mode. Before packing PS gels into a column, fines were first removed as explained in the previous paper [1].

The recommended column packing procedure is as follows. The slurry solvent was a mixture of toluene and chloroform (50.5/49.5, v/v), which has a similar density to PS gels. About 1-g portion of PS gels was swollen in 10 mL of the solvent for overnight. The slurry was stirred for five minutes with a magnetic stirrer and agitated for two minutes with an ultrasonic cleaner. Three column blanks were connected with the packer. A 1.5-mL aliquot of the slurry solvent was poured into the top of the packer-column asssembly, followed by the dispersed and degassed slurry through a 300-mesh copper sieve in order to remove aggregates. The packer was then filled by the same solvent until filled. The slurry solvent was pumped into the packer at the flow rate of 500 μL/min for 7 h by the down-flow method. After being packed, the columns were removed from the packer-column assembly and were equilibrated by pumping tetrahydrofuran (THF) at 40 μL/min for 3 h.

Several other packing procedures such as packing under the constant pressure and packing with viscous slurry solvents were also attempted. The procedures and the results will be discussed at the section of RESULTS AND DISCUSSION.

Column efficiency was evaluated by measuring the number of theoretical plates (N). The values of N were determined by injecting 1 μL of 0.5% benzene solution at a flow rate of 40 μL/min. The mobile phase was THF. Detection was made at 254 nm with an ultraviolet detector Model UVIDEC-100II which holds a semimicro flow cell (0.5 mm i.d. x 5 mm length; cell volume 1 μL). The value of N was calculated by measuring the width of the peak at half height. Oligostyrene A-300 (average molecular weight is 300) (Toyo Soda Mfg. Co., Japan) was used as a test sample for checking the efficiency of the columns.

RESULTS AND DISCUSSION

By the recommended packing procedure, a high-quality column such as 8600 plates/25 cm (HETP = 29.1 μm) has been obtained. The value of N is comparable to conventional SEC (guaranteed maximum HETP = 31 μm) [4]. The chromatogram of benzene obtained on the column is shown in Figure 1. A symmetrical peak with very small tailing has been obtained.

Sixteen columns which have the values of N between 5800 and 10000 were connected in series and used for oligomer analysis [3]. The calculated overall number of theoretical plates was 107000 plates/4 m and the observed one was obtained as 103000 (HETP = 38.8 μm) by injecting 4 μL of 1% benzene solution. The chromatogram of benzene on the combined column assembly is also shown in Figure 1. A typical example of oligomer separation on this column assembly is shown in Figure 2. A 0.5% solution of oligostyrene A-300 was prepared and the injection volume was 4 μL. Seven peaks can be clearly observed.

In the previous paper [1], we revealed that a constant-flow technique is preferable to a constant-pressure technique and that optimum packing flow rate lay between 300 and 350 μL/min. In the experiment in this paper, we tried first to pack PS gels into a single column under a constant-flow technique as described in the previous paper. The result is shown in Figure 3 which seems to elucidate that lower flow rate such as 200 μL/min is advisable. However, when two column blanks were packed together in order to prepare many packed columns in a short period, optimum packing flow rate was higher than 200 μL/min, and that the second column, which was situated at the outlet of the packer-column assembly, had sometimes higher value of N. The results are listed in Table 1. This observation enforced to attempt packing PS gels into three column blanks at the same time.

Figure 4 shows the relationship between packing flow rate and column efficiency when three column blanks were connected in series

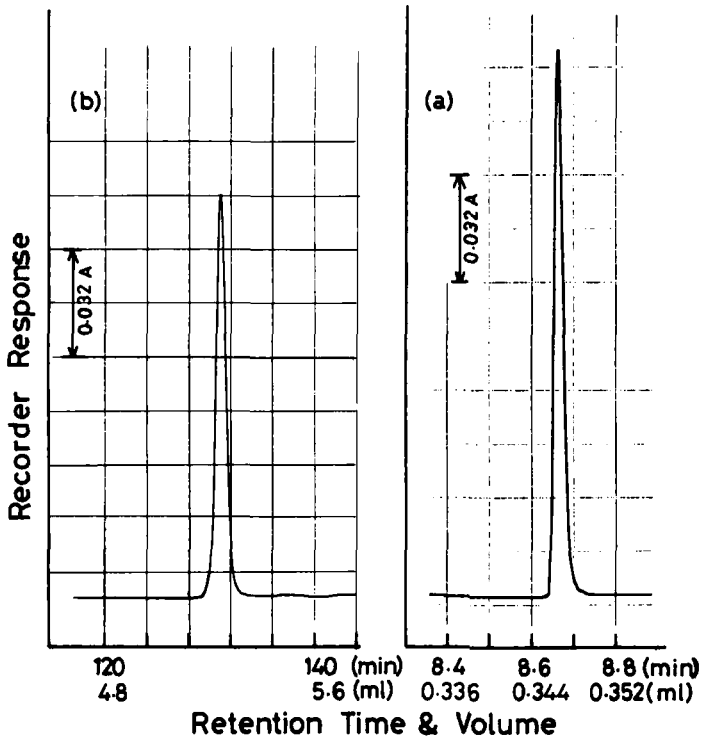

FIGURE 1. Semimicro SEC chromatograms of benzene. Mobile phase: THF; detector: UV at 254 nm; flow rate: 40 μL/min.
(a) On one column (25 cm length). Sample: 0.5% benzene 1 μL; N: 8600 plates/25 cm; attenuation: x0.32 AUFS.
(b) On the combined column assembly (4 m length). Sample: 1% benzene 4 μL; N: 103000 plates/4m; attenuation: x0.32 AUFS.

TABLE 1
Packing Efficiency at Two-Columns Packing Under Constant-Packing Flow Rate

Flow rate μL/min	Final packing pressure Kg/cm^2	N	
		First column	Second column
200	40	2500	1800
250	48	5500	5300
300	63	4700	4900
350	72	5300	5400
400	80	5000	6800
450	90	4400	4200
500	100	3400	5800

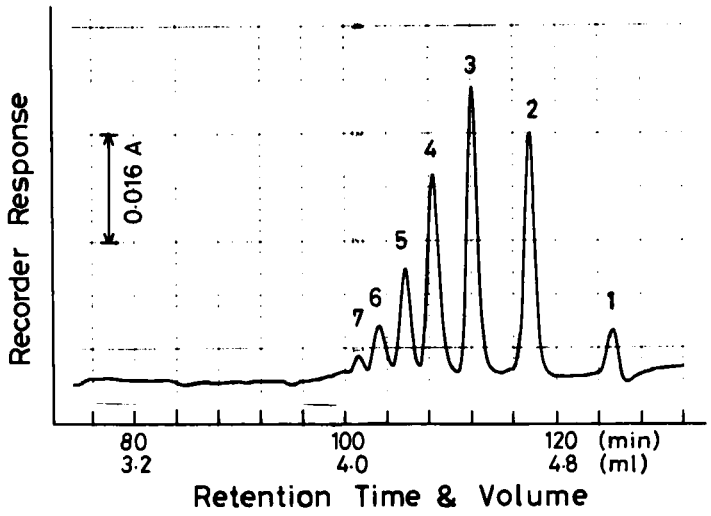

FIGURE 2. Semimicro SEC chromatograms of oligostyrene A-300 on the combined column assembly. Sample: 0.5% 4 μL; flow rate: 40 μL/min; pressure: 50 - 60 Kg/cm^2; detector: UV at 254 nm, x0.16 AUFS.

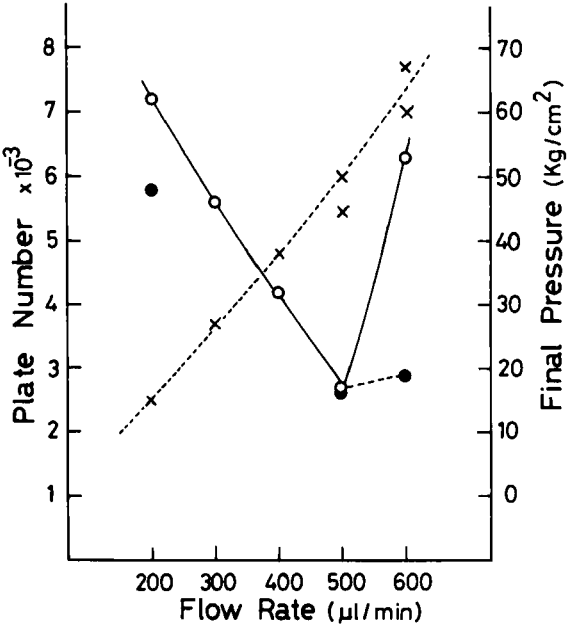

FIGURE 3. Relationship between packing flow rate and the number of theoretical plates in single column packing. (The mark ● means the data of repacking.)

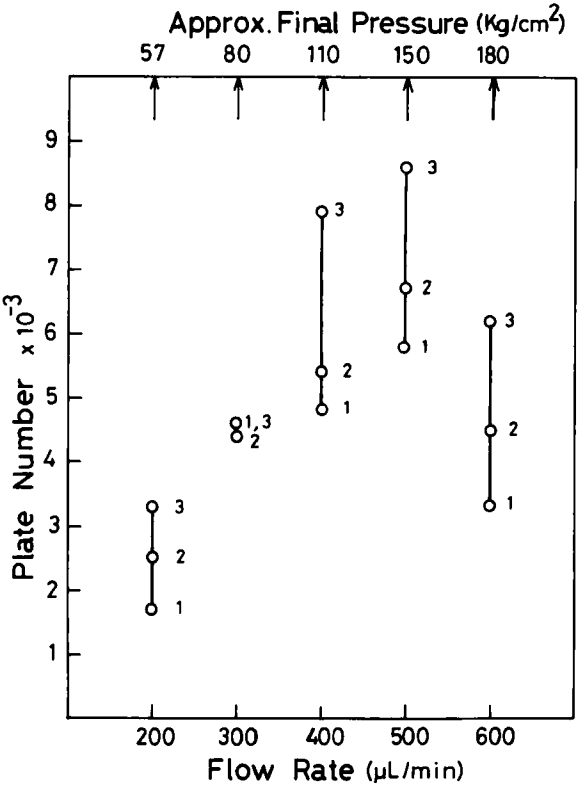

FIGURE 4. Relationship between packing flow rate and the number of theoretical plates when three column blanks were packed at the same time. Numerals in the drawing mean the order of connection of columns to the packer-column assembly from the packer side to the outlet.

and PS gels were packed into them. Optimum flow rate lay between 400 and 500 μL/min, the latter being better than the former. The third column, which was located at the outlet of the packer-column assembly, had always the highest value of the three and the second column was the next. Reproducibility of column was checked and was listed in Table 2. We kept columns which had the value of N higher than 5800 for the further experiment. Therefore, packing efficiency, that means the probability of getting valid columns, can

be said to be about 60%. The packed columns were stored by dipping one end of the column into a THF bottle until use.

Pressure monitoring in progress of packing is very effective to foresee the column efficiency. A smooth and rapid rise in pressure to the prescribed one (fifteen minutes when packing flow rate was 500 μL/min and three column blanks were packed at the same time) and then holding the pressure constant (150 Kg/cm^2 in this case) result in producing high-quality columns. The results are shown in Figure 5. The values of N in the cases of a, b, and c were as follows:

	a	b	c
First column	5800	4250	3100
Second column	6700	5250	4500
Third column	8600	4100	4850

Packing under the constant pressure showed lower values of N than those under the constant flow. The results are listed in Table 3. The value of N, 10000, when packing pressure was 30 Kg/cm^2 was probably obtained by accident.

TABLE 2
Reproducibility at Three-Columns Packing Under Flow Rate of 500 μL/min

Packing No.	N		
	First	Second	Third
1	5800	7700	8400
2	5800	6700	8600
3	4600	5500	6300
4	3100	4500	4900
5	4500	5000	6100
6	4700	6300	6100
7	4600	6000	6400
mean	4700	6000	6700

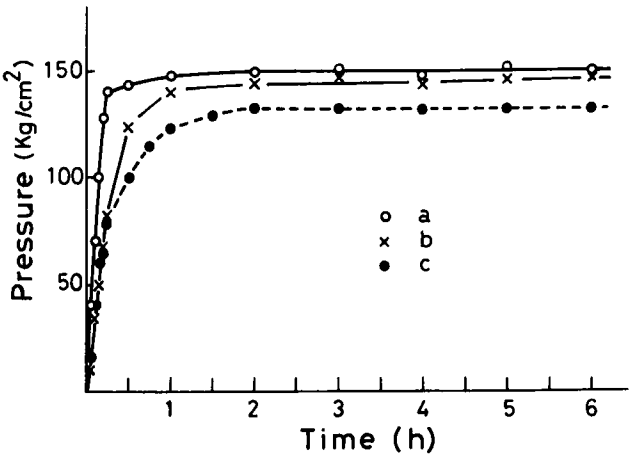

FIGURE 5. Pressure monitoring in progress of packing. For a, b, and c, see text. Packing flow rate: 500 μL/min; three column blanks were connected to the packer-column assembly.

TABLE 3
Packing Efficiency at Two-Columns Packing Under Constant Packing Pressure

Packing pressure Kg/cm²	Final flow rate μL/min	N	
		First column	Second column
30	160	3300	10000
		4400	5400
40	210	5800	5900
		5200	2400
60	330	4900	2200
80	440	4700	5000

Tables 1 and 3 and Figures 3 and 4 indicate that laminar flow of slurry during packing (lower flow rate might be preferable) and higher packing pressure to get more compact gel bed in the column are two typical optimum column packing conditions. Therefore, to pack columns using viscous slurry solvents was supposed to be a valuable technique to get high quality packed columns. For this purpose, polyethylene glycol (PEG) 600 was added to the mixture of toluene and chloroform to obtain a viscous slurry solvent and PS gels were packed into columns under constant flow rate. Density of the mixture was adjusted to be the similar value to PS gels in all cases. Results are shown in Table 4. Unfortunately, we could not get expected results in this case.

In order to get compact gel bed in a column, the other possible technique was to pack gels in the unswollen state and then to swell them in a column. n-Hexane was added in the slurry solvent, the density of the slurry solvent being kept constant. After packing, the solvent in the column was replaced by changing the composition of the mobile phase from the slurry solvent to the mixture of 70% of the slurry solvent and 30% of THF, to the mixture of 40% of the slurry solvent and 60% of THF, and finally to THF. The results are shown in Table 5. Some are good and others are not. It is hard to discuss validity of this method from these results only.

TABLE 4
Packing Efficiency at Two-Columns Packing with Viscous Slurry Solvents Under Constant Flow Rate

Flow rate μL/min	Final pressure Kg/cm^2	Solvent composition (vol%)			N	
		PEG600	Toluene	$CHCl_3$	First Column	Second Column
500	120	5	49.5	45.5	4300	5700
310	120	10	46.4	43.6	3500	4300
220	120	15	43.4	41.6	2300	1900

TABLE 5
Packing Efficiency of Columns Packed in the less Swollen State under Constant Flow Rate of 400 μL/min

Solvent composition (vol%)			Final packing pressure Kg/cm^2	N	
n-Hexane	Toluene	$CHCl_3$		First column	Second column
10	39	51	60	4800	5700
20	26	54	57	4700	5900
30	12	58	52	5000	7000
40	0	60	45	3800	4400

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