14. Perrin, M.; Lecocq, S. *J. Incl. Phenom.* **1991**, *11*, 171.
15. Ninagawa, A.; Matsuda, H. *Makromol. Chem. Rapid Commun.* **1982**, *3*, 65.
16. Steward, D. R.; Gutsche, C. D. *Org. Prep. Proc. Int.* **1993**, *25*, 137.
17. Souley, B.; Safari, Z.; Vicens, J. *Polish J. Chem.* **1993**, *25*, 137.
18. No, K. H.; Hwang, K. L. *Bull. Korean Chem. Soc.* **1993**, *67*, 763.
19. Still, W. C.; Kahn, M.; Mitra, M. *J. Org. Chem.* **1978**, *43*, 2923.
20. No, K. H. Ph.D thesis, Washington University, St. Louis, Mo. 1981.
21. Stewart, D. R.; Krawies, M.; Kashyap, R. P.; Watson, W. H.; Gutsche, C. D. *J. Am. Chem. Soc.* **1995**, *117*, 586.

Improved Glass-Lined Stainless Steel Packed Microcolumns of 0.3 mm I.D.

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Scott and Kucera,[1,2] Tsuda and Novotny[3,4] and Ishii and coworkers[4-7] were the pioneers of microcolumn liquid chromatography. Techniques and designs relevant to microcolumn liquid chromatography have been continuously improved[8-21] since their work in late seventies. The necessary components of microcolumn liquid chromatography, that is, injectors with a very small sample loop, detectors with a very small flow cell, micro-flow pumps, and appropriate fittings are now commercially available.

Recently, most of the microcolumn separation tend to utilize commercial or home-made packed silica capillary columns.[22-37] On the other hand, we have tried to make use of packed glass-lined stainless steel microcolumns in our laboratory.[38-41] It seems that a mirror-like inner surface of the tubing is essential to secure good packing especially when the column diameter is smaller,[39] thus use of glass-lined stainless steel tubing is justified. The merit of glass-lined stainless steel tubing over silica capillaries is its solidity and convenience of handling. Very good care should be taken to prepare and use packed silica capillary columns because of their fragility.

So far, we have obtained numbers of theoretical plates of *ca.* 20,000 for columns of 0.5 mm I.D. (30 cm length),[41] and 10,000 for columns of 0.3 mm I.D.[40] In this study, we have improved the column packing procedure, and have achieved numbers of theoretical plates of 20,000 for columns of 0.3 mm I.D.

Experimental

A Shimadzu (Tokyo, Japan) 10AD pump, an Isco (Licoln, USA) CV4 capillary window detector, a Valco (Houston, USA) Cl4W.05 injector with a 50 nL injection loop, and a Younglin (Seoul, Korea) D520B computing integrator were combined to construct the appropriate micro-LC system.

Methanol was of HPLC grade and obtained from Fisher (Pittsburg, USA) and used without further purification. We chose *p*-nitroaniline, N,N-dimethyl-*o*-nitro-*p*-toluidine, and propylbenzene as the test solutes considering their polarity range and retention times. *p*-Nitroaniline and propylbenzene were purchased from Aldrich (Milwaukee, USA) as reagent grade and used without further purification. N,N-dimethy-*o*-nitro-*p*-toluidine was synthesized in our laboratory.[42]

The Adsorbosphere C18 (5 μ) stationary phase, glass-lined stainless steel tubing, and fitting elements were purchased from Alltech (Deerfield, USA).

We took special care in preparing microcolumns to minimize the extracolumn void volume. The column is directly connected to the injector without any connecting tubing or frit. We prepared a fritted silica tubing (5 cm, 50 μ I.D.) by putting a tiny amount of silica particles at the tip and sintering them on a propane flame,[40] and attached it to the column outlet. The detector optical window was prepared by burning a portion of the polymer coating of a 15 cm silica capillary (50 I.D.) and by introducing the capillary into the cell bolck until the optical window reached the appropriate position. The silica capillary of the column outlet and the silica capillary of the optical window were connected through a glass connector. The extracolumn void volume including the sampling loop of the injector and the inner volume of the transfer line between the column and the detector is estimated *ca.* 0.6 μL. The total mobile phase volume in the column (0.3 mm I.D., 30 cm length) is estimated 21.2 μL assuming the overall column porosity is 0.6.

The Alltech (Deerfield, USA) slurry packer was used to make microcolumns. The stationary phase was dried at 90 °C for 4 hours. The slurry was made by mixing 30 mg particles with 2 mL methanol, and was sonicated for 20 min. before packing. The slurry was transfered to the slurry reservoir (1.2 mL), and the pressure of the slurry packer was raised to 14,000 psi instantly. The pressure was maintained for 2 min., and decreased to 10,000 psi, and the final pressure was maintained for 10 min. The reservoir and the column were continuously vibrated while packing.

The chromatograms of the three test solutes were monitored at 254 nm. The eluent was 100% metanol. The flow rates were varied within 0.001-0.01 mL/min. The retention time and peak width at half height of each solute were measured to compute the number and height equivalents of

Table 1. Theoretical plate numbers of three test solutes measured on the glass-lined stainless steel packed microcolumns of 0.3 mm I.D. at various flow rates

Flow rates[a] (mL/min)	Plate numbers (N±s.d.)		
	p-nitroaniline	DN[b]	propylbenzene
0.001	7700±480	14500±570	16700±850
0.002	21700±980	15800±270	19500±730
0.003	18200±640	16200±310	17100±650
0.005	11600±40	12700±290	13100±330
0.008	8700±80	9100±230	9000±120
0.01	7300±60	7500±330	7500±220

[a] 100% Methanol was used as the eluent. [b] N,N-dimethyl-*o*-nitro-*p*-toluidine.

theoretical plates.

Results and Discussion

The calculated and averaged numbers of theoretical plates based on the retention data measured by three columns produced through the identical packing procedure are assembled in Table 1 for the three test solutes at various flow rates. The plot of HETP (Height Equivalent to Theoretical Plate) *vs.* flow rate is shown in Figure 1. The optimum flow rate is found to be 0.002 mL/min., and the number of theoretical plates at the optimum flow rate, 20,000 or so. The chromatogram of the test solutes observed at the optimum flow rate is given in Figure 2. Such column efficiences are almost two-fold improved compared to those of the previous study[40] and are good enough for analyses of most samples encountered in a wide scope of studies.

Such a progress is due to the reformation of the column packing techniques. The excessively high pressure (14,000 psi) packing within the short time (2 min.) interval at the initial stage, in addition to the use of a very small slurry reservoir (1.2 mL), seems to enable uniform high density packing of the stationary phase particles for the bottom half of the column before breakage of particles occurs. It is known by the vendors that 5 μ C18 stationary phase particles can endure up to 8,000 psi. We believe from our experiences that 10,000 psi is safe for 30 min. Thus, maintaining 10,000 psi for 10 min. will not degrade the column but secure complete compact packing for the upper half of the column.

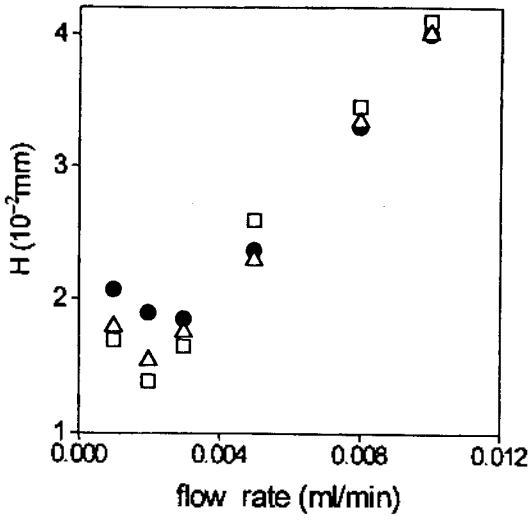

Figure 1. The plot of HETP *vs.* flow rate of the eluent for *p*-nitroaniline(square), N,N-dimethyl-*o*-nitro-*p*-toluidine(circle) and propylbenzene(triangle).

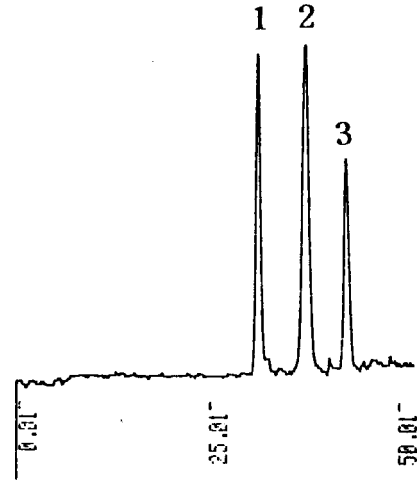

Figure 2. The chromatogram of the test solutes eluted in 100% methanol at 0.002 mL/min., and monitored at 254 nm. 1; *p*-nitroaniline, 2; N,N-dimethyl-*o*-nitro-*p*-toluidine, 3; propylbenzene.

We should note that the use of a glass connector between the silica capillary of the column outlet and the silica capillary of the detector causes a negative effect on column efficiency. Its void volume is approximately 0.3-0.4 μL. Its inner diameter is *ca.* 300 μ but the inner diameter of the capillaries at both sides is 50 μ. Such a connection is apt to cause irregular dispersed flow paths in the connecting area and yield a high band broadening. We suspect that the observation of a minimum in the van Deemter plot (Figure 1) is due to the void volume of the glass connector. In the previous study where we used an identical silica capillary for the column outlet and the optical window without a connector, we were able to observe a monotonous decrease of HETP with decrease of flow rate.[40] The band broadening effect at very slow flow rates seems mostly due to the troublesome element of extracolumn void volume like the glass connector of this study. Nevertheless, use of an identical silica capillary for the column outlet tubing and the optical window is extremely incovenient for routine application. The inevitably existing void volume between the column and the detector unit seems to be the limiting factor in separation efficiency of practical microcolumn liquid chromatography.

Conclusion

We have successfully packed efficient glass-lined stainless steel microcolumns of 0.3 mm I.D. by the rapid high pressure packing procedure. We were able to obtain numbers of theoretical plates of 20,000 for 30 cm columns. The inevitably existing void volume of the transfer line limits separation efficiency of microcolumn liquid chromatography in the practical sense.

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References

1. Scott, R. P. W.; Kucera, P. *J. Chromatogr.* **1976**, *125*, 251.
2. Scott, R. P. W.; Kucera, P. *J. Chromatogr.* **1978**, *169*, 51.
3. Tsuda, T.; Novotny, M. *Anal. Chem.* **1978**, *50*, 271.
4. Tsuda, T.; Novotny, M. *Anal. Chem.* **1978**, *50*, 632.
5. Ishii, D.; Asai, K.; Hibi, K.; Jonokuchi, T.; Nagaya, M. *J. Chromatogr.* **1977**, *144*, 157.
6. Tsuda, T.; Hibi, K.; Nakanishi, T.; Takeuchi, T.; Ishii, D. *J. Chromatogr.* **1978**, *158*, 227.
7. Ishii, D.; Tsuda, T.; Takeuchi, T. *J. Chromatogr.* **1979**, *185*, 73.
8. Takeuchi, T.; Ishii, D. *J. Chromatogr.* **1981**, *213*, 25.
9. Kucera, P. *Microcolumn High Performance Liquid Chromatography*, Ed.; Elsevier: Amsterdam, 1984.
10. Novotny, M.; Ishii, D. *Microcolumn Separations*; Eds.; Elsevier: Amsterdam, 1985.
11. Scott, R. P. W. *Small Bore Liquid Chromatography Columns*, Ed.; Wiley-Interscience: New York, 1984.
12. Novotny, M. *Anal. Chem.* **1988**, *60*, 500A.
13. Harvey, M. C.; Sterns, S. D. *J. Chromatogr.* **1983**, *21*, 473.
14. Borra, C.; Han, S. M.; Novotny, M. *J. Chromatogr.* **1987**, *385*, 75.
15. Novotny, M.; Alasabdro, M.; Konishi, M. *Anal. Chem.* **1983**, *55*, 2375.
16. Kennedy, R. T.; Jorgenson, J. W. *Anal. Chem.* **1989**, *61*, 1128.
17. Grescentini, G.; Bruner, F.; Mangani, P.; Yafeng, G. *Anal. Chem.* **1988**, *60*, 1659.
18. Karlsson, K. E.; Novotny, M. *Anal. Chem.* **1988**, *60*, 1662.
19. Bauer, H. *Chromatographia* **1989**, *28*, 289.
20. Banks, J. F.; Novotny, M. *J. Microcolumn Sep.* **1990**, *2*, 84.
21. Berry, V.; Van Rossum, P.; Pretorius, V. *J. Liq. Chromatogr.* **1990**, *13*, 391.
22. Saito, Y.; Ohta, H.; Nagashima, H.; Itoh, K.; Jinno, K. *J. Liq. Chromatogr.* **1994**, *17*, 2359.
23. Takeuchi, T.; Miwa, T. *Chromatographia* **1994**, *38*, 555.
24. Takeuchi, T.; Miwa, T. *Anal. Chim. Acta.* **1994**, *292*, 275.
25. Hu, R.; Takeuchi, T.; Jin, J.; Miwa, T. *Anal. Chim. Acta.* **1994**, *295*, 173.
26. Howard, A. L.; Thomas, C. L. B.; Taylor, L. T. *Anal. Chem.* **1994**, *66*, 1432.
27. Straub, R.; Linder, M.; Voyskner, R. D. *Anal. Chem.* **1994**, *66*, 3651.
28. Takeuchi, T.; Miwa, T.; Hu, R.; Chu, J. *J. High Resol. Chromatogr.* **1995**, *18*, 745.
29. Saito, Y.; Ohta, H.; Terasaki, H.; Katoh, Y.; Nagashima, H.; Jinno, K.; Itoh, K. *J. High Resol. Chromatogr.* **1995**, *18*, 569.
30. Chu, J.; Hu, R.; Miwa, T.; Takeuchi, T. *Chromatographia* **1995**, *40*, 379.
31. Saito, Y.; Ohta, H; Nagashima, H.; Itoh, K.; Jinno, K.; Okamoto, M.; Chen, Y.; Luehr, G.; Archer, J. *J. Liq. Chromatogr.* **1995**, *18*, 1897.
32. Takeuchi, T.; Miwa, T. *Chromatographia* **1995**, *40*, 159.
33. Holland, L. A.; Jorgenson, J. W. *Anal. Chem.* **1995**, *67*, 3275.
34. Takeuchi, T.; Miwa, T. *Anal. Chim. Acta* **1995**, *311*, 231.
35. Lapolla, A.; Fedele, D.; Baldo, L.; Aronica, R.; Raverdino, V.; Catinella, S.; Seraglia, R.; Traldi, P. *Rapid Comm. Mass Spectrom.* **1996**, *10*, 178.
36. Munaf, E.; Zein, R.; Takeuchi, T.; Miwa, T. *Chromatographia* **1996**, *43*, 304.
37. Jinno, K.; Okumura, C.; Harada, M.; Saito, Y.; Okamoto, M. *J. Liq. Chromatogr. & Rel. Technol.* **1996**, *19*, 2883.
38. Cheong, W. J.; Cha, K. W.; Choi, J. D. *J. Korean Chem. Soc. (Korean)* **1995**, *39*, 471.
39. An, H. J.; Cheong, W. J. *J. Korean Chem. Soc. (Korean)* **1995**, *39*, 863.
40. Cheong, W. J.; An, H. J. *Bull. Korean Chem. Soc.* **1996**, *17*, 539.
41. An, H. J.; Cheong, W. J. *J. Korean Chem. Soc. (Korean)* **1996**, *40*, 462.
42. Cheong, W. J.; Chun, S. H.; Lee, G. Y. *J. Liq. Chrom. & Rel. Technol.* **1996**, *19*, 277.