Journal of Chromatography, 270 (1983) 332–336
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 16,109

Note

Packing columns with flexible walls for high-performance liquid chromatography

S. C. POWELL and Z. K. SHIHABI*

Department of Pathology, Bowman Gray School of Medicine of Wake Forest University, Winston-Salem, NC 27103 (U.S.A.)

(First received October 20th, 1982; revised manuscript received June 30th, 1983)

Flexible-walled columns used under radial compression have certain advantages over steel columns[1]. These columns do not void or channel, can be operated at high flow-rates while maintaining low pressure and in general produce comparable or slightly better separations than steel columns[2–4].

Different methods have been described for packing steel columns with small-diameter particles[5–9]. However, no methods have been described for packing flexible-walled columns. In this paper, we describe two methods for packing such columns. The first is based on the traditional slurry packing technique; the second is novel in that it uses centrifugal force for packing columns in the dry state. Success with particles as small as 5 μm in diameter was experienced utilizing both column packing techniques.

MATERIALS AND METHODS

Column performance was tested using a high-performance liquid chromatograph system consisting of a Tracor Instruments (Houston, TX, U.S.A.) Model 970A variable-wavelength detector equipped with a Houston Instrument (Austin, TX, U.S.A.) Recordall Series 5,000 recorder, a Waters Assoc. (Milford, MA, U.S.A.) Model U6K injector and a Laboratory Data Control (Riviera Beach, FL, U.S.A.) Constametric I pump.

Flexible-walled tubing supports were prepared by machining polyethylene glycol tubing to fit the Water Assoc. radial compression module, Model RCM-100, or by emptying cartridges supplied commercially for use with the RCM-100. The cartridges were opened most conveniently by wedging off the first housing at both ends with a tight fitting metal rod.

Slurry packing

A Micromeritics (Norcross, GA, U.S.A.) stirred-slurry column packer, Model 705 was used for wet packing. Using an excess of packing material (6 g) suspended and stirred in 25 ml of an equal volume solution of methanol–isopropanol–acetonitrile, columns held in the RCM-100 unit pressurized with lever 1 were packed at a flow-rate of 10 ml/min (pressure $\leqslant$2000 p.s.i.). The flow-rate was decreased as the

0021-9673/83/$03.00

column filled to avoid exceeding the pressure limit. The RCM-100 was used here mainly as a convenient holder for the columns. An inlet with a 1 mm inside diameter was machined to connect between the packer and the RCM unit. Plate number (N) was calculated for β-hydroxyethyltheophylline based on the formula: $N = 16 \cdot (\text{peak retention time})/(\text{width of the peak})^2$.

Dry packing

Empty columns were filled to the top with the desired spherical packing material 4.8 g for 8 mm cartridge under slight vibration and placed in an International Equipment (Needham Hts., MA, U.S.A.) Model K centrifuge equipped with a No. 250 head and centrifuged for 5 min at 1000 *g*. The column was removed, refilled once more if necessary with the same packing material and centrifuged again for 5 min. The columns were washed with a 100 methanol followed by 200 ml pump solvent.

RESULTS AND DISCUSSION

The stirred-slurry technique is commonly used for packing steel columns[5–9]. In the present study, we have adapted the same technique for packing columns with

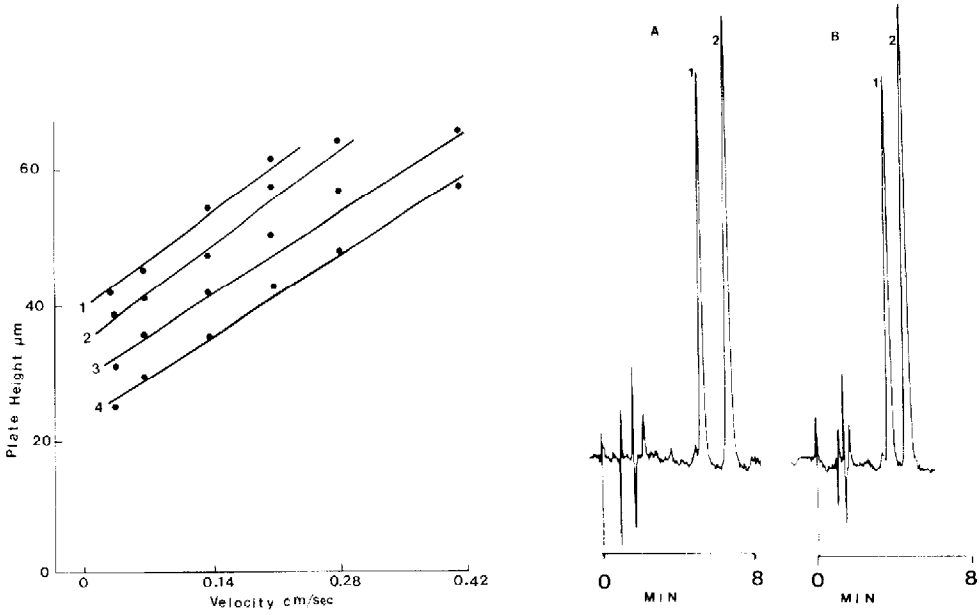

Fig. 1. Plate height (μm) *versus* linear velocity cm/sec: (1) RoSil 8 μm dry packed (8-mm cartridge); (2) RoSil 8 μm slurry packed (5-mm cartridge); (3) RoSil 8 μm slurry packed (8-mm cartridge); (4) Waters Assoc. 10 μm (8-mm cartridge); commercially packed.

Fig. 2. Comparison of (A) slurry packed column, RoSil C_{18}, 5 μm (Alltech Assoc., Deerfield, IL, U.S.A.) to (B) commercial column, C_{18}, 5 μm (Waters Assoc.). Peaks: 1 = theophylline; 2 = β-hydroxyethyltheophylline. Chromatographic conditions: mobile phase, 9.5% acetonitrile in acetate buffer 26 mmol/l; flow-rate 1.5 ml/min; detector wavelength 275 nm; and attenuation 0.020 A.

flexible walls to be used under radial compression. Columns prepared in this manner were found to be comparable in their separations to commercially prepared columns having achievable peak asymmetry $\leqslant 1.2$ and plate numbers of 40,000–60,000 plates per meter for the 5-μm particles (Figs. 1 and 2). Plate number decreased as the linear velocity increased, but it did not change with changing sample size between 4 and 80 μl. Columns packed with 5 μm were found to have low back pressure and could be operated at flow-rates of 5.0 ml/min with pressures about 2000 p.s.i. Both the spherical and the non-spherical particles were packed by the slurry packing technique, and a wide variety of packing materials, including some not commercially available at the present time in prepacked flexible-walled columns were utilized. As can be seen in Fig. 3, the 10-cm flexible-walled column packed with ion exchanger had slightly better resolution than the 10-cm steel column using identical packing materials for both columns. Throughout this work, it was our experience that the RCM columns could be packed and repacked using the same material with reproducible results.

It is known that column diameter affects the plate number for steel columns[3]. In general, rigid walled columns of 5 mm I.D., for example, generate higher plate numbers than columns with 1 to 3 mm I.D. The effect becomes negligible over 5 mm in diameter. It can be seen (Figs. 2 and 4) that the plate number for flexible-walled columns is more heavily dependent on column diameter. For the 10-cm RCM col-

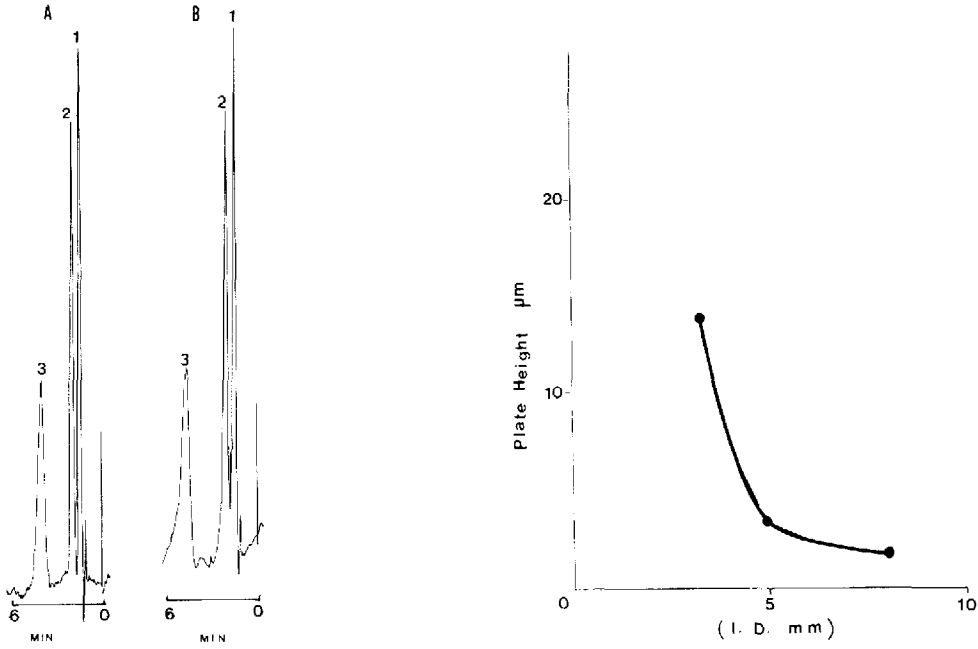

Fig. 3. Comparison of the flexible-wall columns and the steel columns both packed by slurry packing: (A) flexible-wall column 100 × 5.0 mm I.D.; (B) steel column 100 × 4.6 mm I.D. Peaks: 1 = adenosine 5'-monophosphate; 2 = adenosine 5'-diphosphate; 3 = adenosine 5'-triphosphate. Chromatographic conditions: solvent 250 mmol/l phosphate buffer, pH 6.0; linear velocity 0.10 cm/sec and wavelength 260 nm.

Fig. 4. Relationship between plate height and column diameter for the flexible-wall columns at 0.10 cm/sec linear velocity. Packing, RoSil C_{18} 5 μm.

umn, the number of plates per meter for the 2.75, 5.0 and 8.0 mm I.D. columns was 800, 28,300 and 50,100, respectively, at the same linear velocity. We do not have an explanation for the fact that the plate number drops dramatically for small-diameter columns; possible the extra-column effects on band broadening become critical with the smaller internal diameter columns. The narrow columns exhibited higher sensitivity as expected.

A second major factor in column performance is particle size uniformity[5]. We found that by removing the fine material from the column packing we would, in some cases, significantely improve the plate number. This improvement however, was dependent upon the source of the packing material. The Spherisorb S5 ODS2 and the RoSil C_{18} 8 μm was the easiest to pack, probably due to a narrow distribution of particle size in these packing materials.

Although the slurry packing technique yields good columns, it requires special instrumentation, time and skill. Columns have to be packed one at a time. In many cases, this is not applicable for the average laboratory. It would be more desirable to pack columns in the dry form. However, it is accepted that particles less than 20 μm in diameter cannot be packed dry by the tap and shake methods used for larger particles.[5] In a novel approach, we have found that centrifugal force can be used where other methods have failed to pack small particles (< 20 μm). Using centrifugation, spherical particles 5 and 8 μm in diameter were successfully packed in RCM columns with plate numbers of approximately 30,000 per meter (Figs. 1 and 5). The 8-μm particles were easier to pack than the 5-μm particles. Centrifugal force for packing columns has not been described before. Although the slurry packing tech-

Fig. 5. Separation of theophylline (1) and β-hydroxyethyltheophilline (2) on RoSil C_{18} packing, 8-μm particles packed dry by centrifugation. Chromatographic conditions as in Fig. 2.

nique yields columns with higher plate number, the simplicity of the centrifugal force packing and the potential ability to pack many columns simultaneously render the latter method worthy of further studies. Different periods of centrifugation and different centrifugal forces as well as pretreating the particles with agents which decrease their coulombic attraction[9] might need further study to improve the plate height by this method. Irregular particles did not yield useful columns by dry packing.

REFERENCES

1 H. C. Jordi, U. D. Neue, H. M. Quinn and C. W. Raush, in G. L. Hawk (Editor), *Biological/Biomedical Applications of Liquid Chromatography III* (*Chromatographic Science Series*, Vol. 18), Marcel Dekker, New York, 1980, p. 327.
2 S. P. Assenza and P. R. Brown, *J. Liquid Chromatogr.*, 3 (1980) 41.
3 S. J. Soldin, *Clin. Biochem.*, 2 (1980) 99.
4 J. S. Landy, J. L. Ward and J. G. Dorsey, *J. Chromatogr. Sci.*, 21 (1983) 49.
5 L. R. Snyder and J. J. Kirkland (Editors), *Introduction to Modern Liquid Chromatography*, Wiley, New York, 2nd ed., 1979. Ch. 5, p. 168.
6 R. M. Cassidy and D. S. LeGay, *Anal. Chem.*, 46 (1974) 340.
7 C. J. Little, A. D. Dale, D. A. Ord and T. R. Marten, *Anal. Chem.*, 49 (1977) 1311.
8 M. Broquaire, *J. Chromatogr.*, 170 (1979) 43.
9 J. C. Liao and J. L. Ponzo, *J. Chromatogr. Sci.*, 20 (1982) 14.