Journal of Chromatography, 264 (1983) 258–263
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 15,844

Note

Effect on column efficiency of packing capillary columns with inert spheres for use in liquid chromatography

HERNAN J. CORTES
The Dow Chemical Company, Analytical Laboratories, Midland, MI (U.S.A.)
(Received March 8th, 1983)

The limiting theoretical efficiency in liquid chromatography (LC) was defined by Giddings[1] as

$$N_{\text{lim}} = \frac{d_p^2 \, \Delta P}{4 \, \psi \, \gamma \, \eta \, D_m} \tag{1}$$

Where d_p = particle size, ΔP = pressure drop, ψ and γ = geometrical constants, η = viscosity and D_m = diffusional coefficient in the mobile phase.

Recent trends intended to increase theoretical plate count and speed of analysis in LC have centered on reducing particle size to a few micrometers[2], reducing column diameter in what are termed microbore columns[3,4] and using open tubular columns, analogous to capillary gas chromatography[5–7]. Due to high pressure drops encountered with a decrease in particle size, it is unlikely that significant advances can be made by reducing particle size below 3 μm.

Microbore columns have found utility; however, the limit in efficiency is determined by the eddy diffusion found in microparticulate packed columns. Open tubular columns offer an alternative to packed column liquid chromatography, and as shown by the Taylor equation[8] for an unretained component:

$$\frac{H}{\mu} = \frac{r^2}{24 \, D_m} \tag{2}$$

where H = theoretical plate height and μ = linear velocity.

It is evident that a decrease in capillary radius (r), and/or an increase in the diffusion coefficient in the mobile phase (D_m) should lead to a higher theoretical plate count. For capillary LC to compete with packed-column LC, Yang[9] indicated that

$$(h/v)_c \, d_c^2 < (h/v)_p \, d_p^2 \tag{3}$$

where h = reduced plate height, v = reduced velocity and d_c = capillary diameter.

Thus, capillaries of 10 μm or less are apparently necessary. The problems encountered with extra-column effects in the injector, detector and connections become considerable with such small columns.

0021-9673/83/$03.00

Various approaches have been taken in the attempt to utilize relatively large (100–250 μm) capillary columns, such as the introduction of secondary flow (radial redistribution of components across a capillary) by tightly coiling a column[10] or using geometrically deformed open tubes[11]. The present work describes the effect of packing 250-μm I.D. capillary columns with 175-μm glass beads. Such an approach proved effective in reducing band spreading in hollow fiber suppressors for ion chromatography[12].

EXPERIMENTAL

Chemicals

Chemicals used were reagent grade, available from Aldrich (Milwaukee, WI, U.S.A.). Solvents used were distilled in glass, available from Burdick & Jackson Labs. (Muskegon, MI, U.S.A.).

Liquid chromatography

The LC system consisted of a Jasco UVIDEC 100-II detector equipped with a modified cell[13], whose volume was calculated as 48 nl from the capillary diameter and the slit size. Wavelength used was 235 nm at 0.005 a.u.f.s.

A Valco injector was used in the split injection mode, where the tip of the capillary column was used as the splitter, and a needle valve controlled the flow into the columns. The pump used was a Waters Assoc. Model M-6000, at a flow-rate of 1.0 ml/min. The eluent throughout this work was hexane with 0.07% methanol.

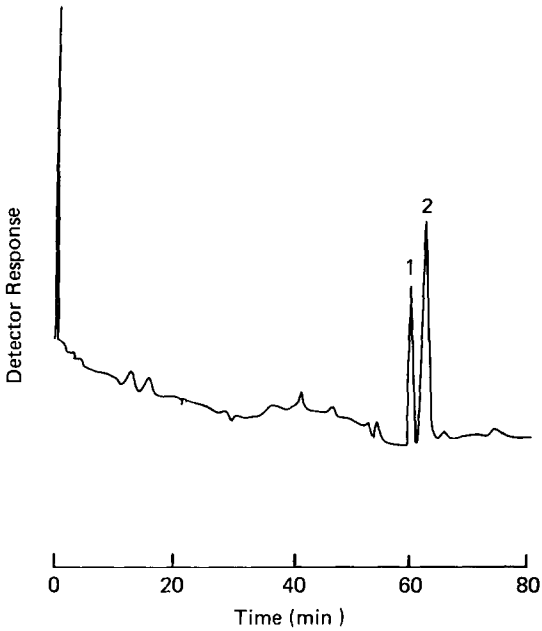

Fig. 1. Separation of nitrobenzene (1) and acetophenone (2) on a 5 m × 250 μm I.D. column. Eluent, hexane–methanol (99.93:0.07); flow-rate, 1.2 μl/min; detector, Jasco Uvidec 100-II at 235 nm; split ratio, 1000:1. Injected components were prepared to give concentrations of 1000 μg/ml.

Columns

Columns were prepared from soft glass by drawing 0.25 in. O.D. × 1 mm I.D. tubes in a glass-drawing machine. Column dimensions were 0.25 mm I.D. × 0.80 mm O.D. after drawing. The surface of the capillary was treated with 1 *N* sodium hydroxide solution for 48 h at 30°C, as reported by Ishii *et al.*[14], and then washed with methanol and methanol–hexane.

The column was packed with 175-μm glass beads available from Duke Scientific (Palo Alto, CA, U.S.A.), by applying vacuum and drawing the beads while the column was immersed in an ultrasonic bath.

RESULTS AND DISCUSSION

A 5-m sodium hydroxide-treated column was evaluated by injecting a mixture of nitrobenzene and acetophenone. A separation was obtained at the conditions listed in Fig. 1. Plate heights obtained as a function of linear velocity for nitrobenzene, the unretained component, are plotted in Fig. 2. The theoretical plate heights calculated from the Taylor equation were also plotted and are included for comparison, indicating an acceptable agreement. The same column was then packed with 175-μm glass beads as described in the Experimental section and evaluated in a similar manner (Fig. 3).

Comparing Figs. 1 and 3, it is evident that both the unretained and retained components are sharper in Fig. 1, the unpacked capillary. We expected to obtain sharper peaks in the packed capillary due to the introduction of secondary flow caused by the packing.

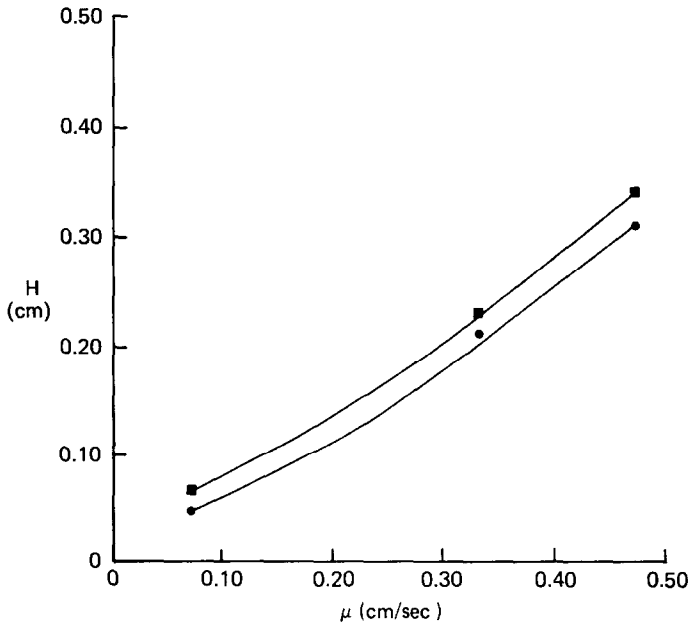

Fig. 2. Plot of plate height *vs.* linear velocity for open tubular 250-μm I.D. column; ● = theoretical; ■ = experimental.

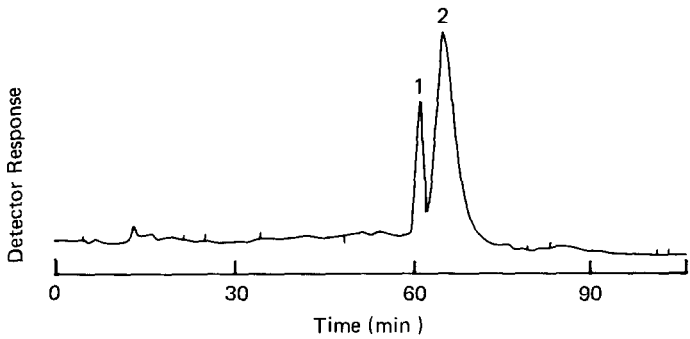

Fig. 3. Separation of nitrobenzene (1) and acetophenone (2) on 5 m × 250 μm I.D. column packed with 175-μm spheres. Conditions as in Fig. 1.

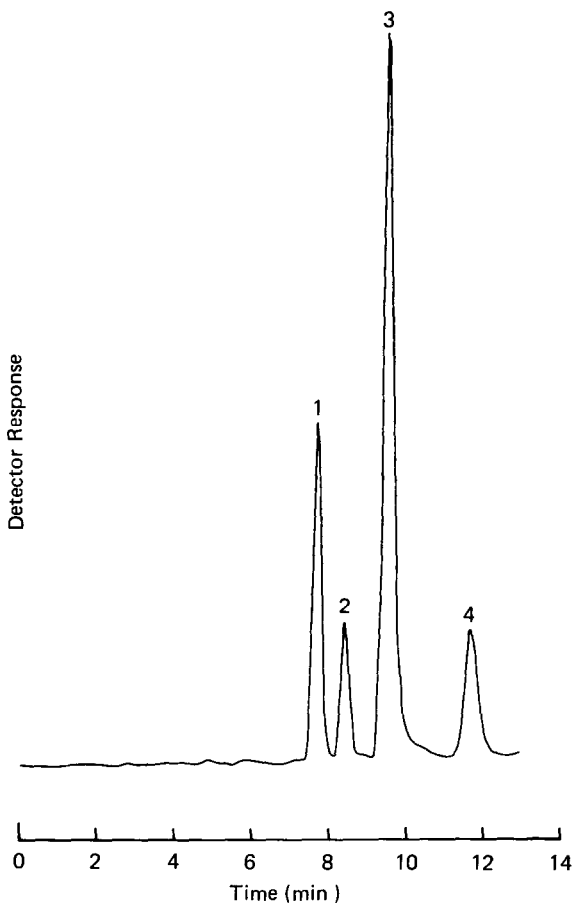

Fig. 4. Separation of pentamethylbenzene (1), nitrobenzene (2), benzaldehyde (3), and acetophenone (4) on 325 cm × 50 μm I.D. column. Conditions as in Fig. 1, except flow-rate, 0.83 μl/min.

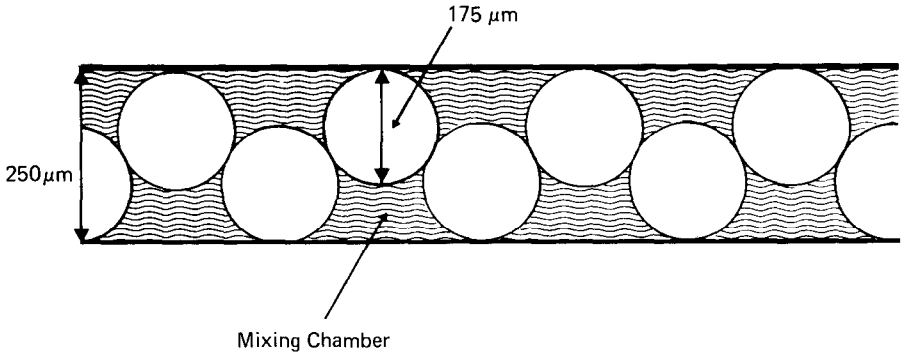

Fig. 5. Expanded view of capillary column packed with spheres.

Hofmann and Halász[11] reported a sharpening of unretained peaks and a broadening of retained peaks for geometrically deformed capillary columns. They attributed the phenomena to lack of a satisfactory method of coating a stationary phase on the capillary surface. In order to determine if that was the case in our system, a 50-μm I.D. column was treated as previously described and evaluated by injecting a mixture of pentamethylbenzene, nitrobenzene, benzaldehyde, and acetophenone (Fig. 4). The 2–10-fold decrease in efficiency of retained peaks described[11] was not observed, indicating a satisfactory coating technique.

We offer an alternate hypothesis: the flow velocity profile across an ideal capillary operated under laminar flow conditions is a parabola, and results in band broadening of eluting peaks. Creating secondary flow, *i.e.* radial mixing in a capillary, should reduce band broadening by radially redistributing the components across the capillary; however, in a system with inert spheres of *ca.* 0.7 capillary diameter size, voids exist between the stationary phase surface and the contact point of the bead (Fig. 5). At the linear velocities used, these voids can act as mixing chambers, described as the "tanks in series model"[15] by

$$c = \frac{1}{t'}\left(\frac{t}{t'}\right)^{N-1} \frac{1}{(N-1)!} e^{-t/t'} \tag{4}$$

where c = observed concentration, t' = mean residence time, N = number of mixing chambers, t = injection time. Thus, at large values of N, the variance observed on an eluting peak can be described as

$$\sigma^2 = Nt'^2 \tag{5}$$

explaining the results obtained.

CONCLUSIONS

The use of inert beads to induce secondary flow in capillary liquid chromatography does not increase theoretical plate count due to mixing chamber effects.

Both retained and unretained components experienced band-broadening when injected into 250-μm capillaries packed with 175-μm spheres. Although an improvement is seen on large-bore tubes[12], at smaller diameters open tubes appear to be more efficient.

ACKNOWLEDGEMENTS

The author thanks T. Stevens and G. Jewett for their helpful discussions.

REFERENCES

1 J. Giddings, *Anal. Chem.*, 36 (1964) 1890.
2 J. DiCesare, M. Dong and L. Ettre, *Chromatographia*, 14 (1981) 257.
3 R. P. W. Scott and P. Kucera, *J. Chromatogr.*, 169 (1979) 51.
4 R. P. W. Scott, P. Kucera and M. Munroe, *J. Chromatogr.*, 186 (1979) 475.
5 T. Tsuda and M. Novotny, *Anal. Chem.*, 50 (1978) 271.
6 T. Tsuda, K. Hibi, T. Nakanishi, T. Takeuchi and D. Ishii, *J. Chromatogr.*, 158 (1978) 227.
7 K. Hibi, T. Tsuda, T. Takeuchi, T. Nakanishi and D. Ishii, *J. Chromatogr.*, 175 (1979) 105.
8 G. Taylor, *Proc. Roy. Soc. London, Ser. A*, 219 (1953) 183.
9 F. J. Yang, *J. Chromatogr. Sci.*, 20 (1982) 241.
10 R. Tijssen, *J. Separ. Sci. Technol.*, 13 (1978) 681.
11 K. Hofmann and I. Halász, *J. Chromatogr.*, 199 (1980) 3.
12 T. Stevens, G. Jewett and R. Bredeweg, *Anal. Chem.*, 54 (1982) 1206.
13 F. Yang, *J. Chromatogr.*, 236 (1982) 265.
14 D. Ishii, T. Tsuda and T. Takeuchi, *J. Chromatogr.*, 185 (1979) 73.
15 J. Ruzicka and E. Hansen, *Flow Injection Analysis*, Wiley, New York, 1981.