Journal of Chromatography, 239 (1982) 507–513
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 14,526

PACKED MICROCAPILLARY LIQUID CHROMATOGRAPHY WITH REDUCED I.D. COLUMNS

TAKAO TSUDA*, ISAO TANAKA and GENKICHI NAKAGAWA
Laboratory of Analytical Chemistry, Nagoya Institute of Technology, Gokiso-cho, Showa-ku, Nagoya-shi 466 (Japan)

SUMMARY

Packed microcapillary columns with internal diameters and particle size (silica gel) 30–40 μm and 10 μm, respectively, were prepared with the aim of reducing analysis time. The *h*–*v* relation for this microcapillary is similar to that of the microcapillary of 75 μm I.D. and particle size 30 μm, which has hitherto been thought to be the optimum. Initial results show that analysis time would be reduced to one-fifth. Applications for aromatic compounds were demonstrated. Combined loop and split injection was used. The reproducibility is good, and the operation simple.

INTRODUCTION

One of the recent topics in the field of liquid chromatography (LC) has been the development of micro columns[1–12], because they have potentially higher resolution than ordinary columns, such as 2–4 mm I.D. and 25 cm length. There are three different types of micro columns: micro-bore packed column[1,2], packed microcapillary column[3–6] and open-tubular microcapillary column[7–10]. The cross-sectional area of the micro-bore packed column is reduced to *ca.* 10% compared with that of an ordinary column, but the cross-sectional areas of microcapillary columns are even smaller, 0.1% or less.

The techniques[1,2] used for the micro-bore packed column, such as column packing method, sample introduction, detection and connection systems, are similar to those of ordinary LC. A micro-bore packed column with 250,000 plates has been demonstrated[1]. Because the column size reduction in microcapillary LC, is so striking, techniques and operational procedures are very different from ordinary LC. These have been investigated during past four years[3–10]. It is apparent from experimental results that capillary LC has the ability to give high resolution in the analysis of complex mixture[3–10].

One of the favourable characteristics of packed microcapillary LC compared with open-tubular microcapillary LC is that the sample capacity is larger because the column contains supports, such as silica gel or activated alumina, which have a large surface area. But a higher inlet pressure is necessary owing to the low permeability compared with open-tubular microcapillary LC.

0021-9673/82/0000–0000/$02.75

The most severe drawback for both packed and open-tubular microcapillary LC is that it needs a long analysis time to get a fine separation which shows a high number of theoretical plates, *e.g.*, over 100,000[5-8]. For analysis time to be reduced, the key factor might be to reduce the column I.D. as predicted theoretically[11-13]. We have attempted to reduce the I.D. of packed microcapillary column to be less than 50 μm. and we have also combined loop and split injection.

EXPERIMENTAL

Original soda-lime glass capillary tubing (0.2–0.3 mm I.D., 6 mm O.D. and 1 m length) was supplied by Ishizuka Glass (Nagoya, Japan). A glass-drawing machine (GDM-1, Shimadzu Seisakusho, Kyoto, Japan) was used to make packed microcapillaries. UV detector (JVIDEC-100II, Japan Spectroscopic) was used with a home-made microcell and connections that were same as system B of ref. 9.

Preparation of packed microcapillary columns with reduced I.D.

Original soda-lime glass was dry-packed with silica gel, Develosil[R] 10-60 (particle diameter 10 μm and pore size 60 Å) made by Nomura Kagaku (Seto, Aichi-Pref., Japan), and then drawn into packed microcapillary by the glass-drawing machine, which was place in the vertical position[3].

To obtain a good packed microcapillary column that is uniformly packed, it is essential to exclude moisture from the silica gel and the original glass capillary tubing and to prevent any access of moisture from the air throughout the whole procedure. Before dry-packing in original glass, the following procedure is necessary. After the inside wall of the original glass capillary was washed with ethanol, it was dried with dry nitrogen and then dried by a burner under flowing dry nitrogen in the original capillary, and finally dried at 500°C and a flow-rate of *ca.* 2 cm/min by passing through the oven. This was done by using the drawing machine, with the bent pipe part detached. To exclude moisture from the air, one or both ends of the original glass were closed by silicone grease or a silicone tube cap.

Silica gel should be also dried in the oven at 500–700°C for 10–60 min, or at 200°C for 2 days. The dried silica gel was dry-packed into the dried original glass capillary tubing by vibrator action. It took *ca.* 30–60 min. Then the dry-packed original glass tubing was drawn out to a packed microcapillary column. If the inside of the original glass tubing became inflated during drawing, it was because of incomplete drying.

The oven temperature for drawing was also one of the factors influencing uniform packing of the microcapillary column: lower temperatures are preferable. This may be because of the viscosity of the original glass tubing at the point of glass-stretching during drawing.

Preparation of ODS-packed microcapillary column

A microcapillary column with an octadecylsilane stationary phase[4] was prepared by the *in situ* column technique described Gilipin *et al.*[14], as follows. Several coils of microcapillary was filled hexane or toluene, and then 0.2 ml of 2% octadecyltriethoxysilane–xylene solution was passed into the capillary at 110°C. The capillary was then washed with hexane, and the process was repeated two or three

times. Then the capillary was washed with dichloromethane and methanol (or methanol alone for use in the reversed-phase mode).

Loop–split injection

In capillary LC there are currently two methods of sample introduction, namely with[3,9] or without[5,7,15] sample splitting. Although the sample introduction system is an important aspect in the construction of a capillary LC system, neither method has yet achieved predominance. Concerning split injection, the geometrical consideration was examined in a previous report[8]. Here the loop, with an inner volume of 20 μl, was combined via a six-way valve with a split injector, by using short stainless-steel tubing, 5 cm × 0.2 mm I.D.

The reproducibility of loop–split injection and the relationship of height equivalent to theoretical plate (*H*) and split ratio are show in Figs. 1 and 2, for packed and open-tubular microcapillary columns under the following conditions. The I.D. of stainless-steel tubing at the split part and the O.D. of the head of the capillary column were 0.8 mm and *ca.* 0.6 mm, respectively. The split ratio was obtained by measuring the amount of effluent by weight, which was collected both at the column outlet and as a discard flow. The experiment was performed at constant pressure to ensure a constant flow-rate in the column. The sample was 10% benzene in octane, or 0.1% N-alkylanilines, and the eluent was hexane.

The reproducibility of loop–split injection is very good. The standard deviation of the sample amount was *ca.* 2%. From Fig. 2, at lower flow-rates, *ca.* 1 cm/sec, the split ratio should be kept higher than 700, but at higher flow-rates, *ca.* 6 cm/sec, a split ratio of 100 can be used without any effect on the chromatographic peaks.

In other words, there might be necessary at some amount of effluent for clean-

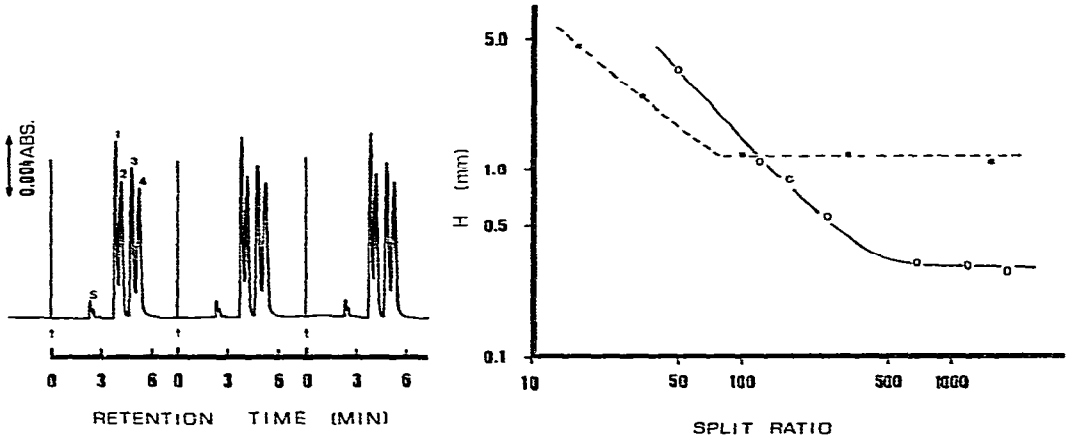

Fig. 1. Reproducibility of loop–split injection. The volume of the sample loop was 20 μl. The sample was N-substituted anilines in octane, at concentrations of 0.1% each. Peaks: S = solvent; 1 = N,N-dimethylaniline; 2 = N-butylaniline; 3 = N-ethylaniline; 4 = N-methylaniline.

Fig. 2. Split ratio *vs. H* for benzene, using loop–split injection method. Curves: O, packed microcapillary column (231 cm × 53 μm I.D.); ■, open-tubular microcapillary column (10 m × 57 μm I.D.). Mobile phase, hexane. Linear velocity is 1.0 cm/sec for packed microcapillary and 6.4 cm/sec for open-tubular microcapillary column.

ing up the part of splitting. If the split ratio is over 1000, the chromatogram is not affected by sample introduction. Although this method of sample introduction requires a larger amount of sample than the direct method, it is very easy to operate.

RESULTS AND DISCUSSION

H–v relation with packed microcapillary column with reduced I.D.

Fig. 3 shows the H–v relation. The H value (height equivalent to a theoretical plate) at 1 cm/sec is nearly 0.2 mm for retained solute (capacity factor, $k' = 0.75$). This value is comparable with the H values for retained solutes in open-tubular microcapillary columns of 30–50 μm I.D.[7,8,10]. In open-tubular microcapillary columns, the mobile-phase flow is strictly laminar flow[7,10], and zone broadening due to the mobile phase can be explained by Taylor diffusion[13]. But in packed microcapillary columns, the zone broadening due to the mobile phase may originate from both Taylor diffusion and eddy diffusion.

As the packed microcapillary column has supports or adsorbents inside the capillary tubing, the inner diameter of the channel in packed microcapillary column might be reduced to one-half or one-third of that of an open-tubular microcapillary column. In the other words, a packed microcapillary column with 30 μm I.D. has almost same size of mobile phase flow channel as an open-tubular microcapillary with 10 μm I.D. From our initial results shown in Fig. 3, the H value is nearly same as that obtained with an open-butular microcapillary with 30–50 μm I.D. This suggests that there may be eddy diffusion in the mobile phase.

h–v relation

The relationship between the reduced plate height (h) and the logarithm of the reduced velocity for retained solute is shown in Fig. 4. Plots of reduced values are

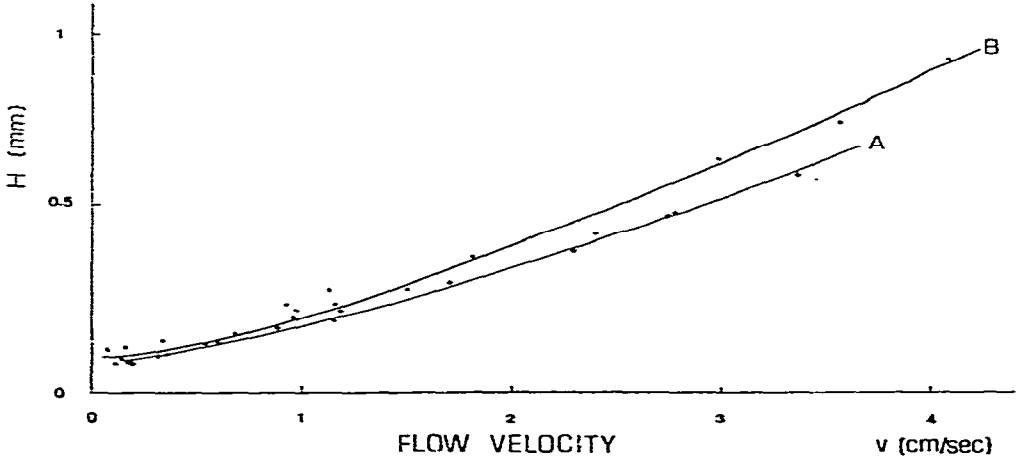

Fig. 3. H vs. flow velocity for packed microcapillary columns. (B) Column 2.2 m × 41 μm I.D.; adsorbent, silica gel, 10 μm particle size; samples, N,N-dimethylaniline ($k' = 0.75$); eluents, 0.12% methanol–hexane. (A) Column 450 cm × 36.5 μm I.D.; adsorbent, silica gel, 10 μm; sample, benzene; eluent as in (B).

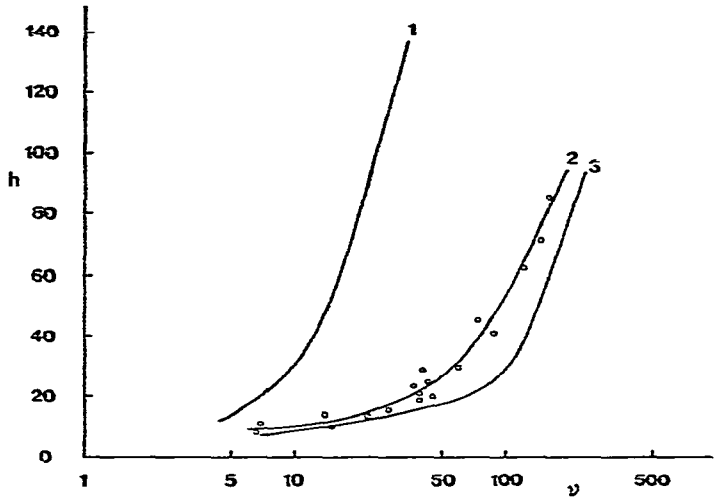

Fig. 4. h–ν relationships for different packed microcapillary columns. (1) Column, 3.6 m × 50 μm I.D.; adsorbent, alumina, 10 μm; sample, pyridine (retention relative to benzene, 0.1); eluent, 0.03% methanol–hexane. (2) Column, 2.2 m × 41 μm I.D.; conditions as in Fig. 3(A). (3) Column, 14 m × 75 μm I.D.; adsorbent, alumina, 30 μm; sample, quinoline (retention relative to benzene, 1.7); eluent, 0.03% methanol–hexane. Curves 1 and 3 quoted from ref. 3.

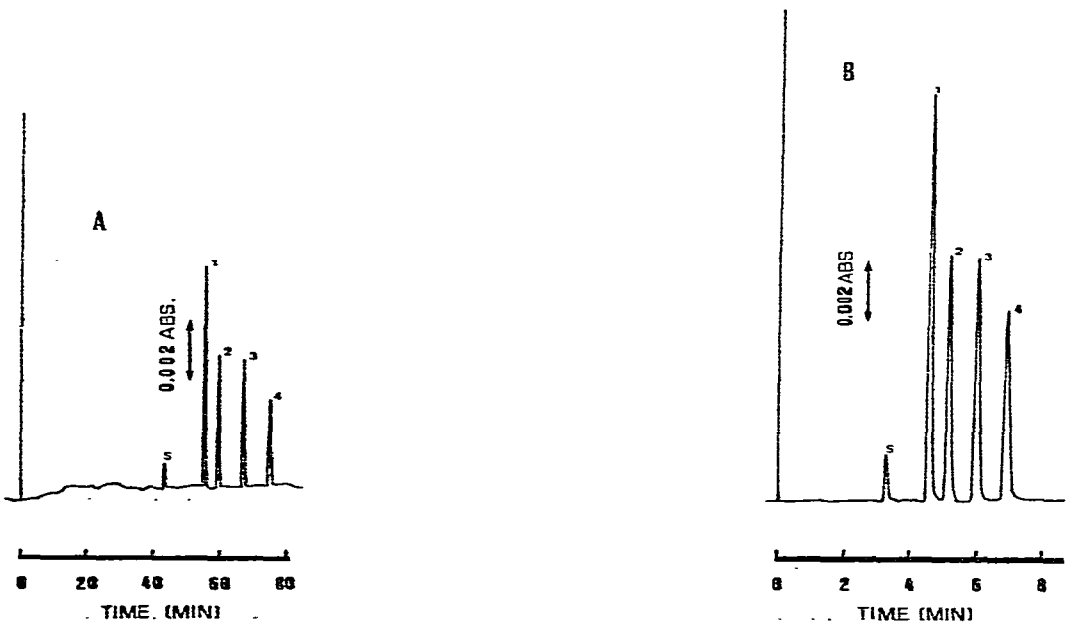

Fig. 5. Separation of N-substituted anilines on silica microcapillary columns. (A) Column, 8 m × 41 μm I.D.; adsorbent, silica gel, 10 μm; mobile phase, hexane with 0.3% methanol; linear velocity, 0.3 cm/sec; inlet pressure, 40 kg/cm^2; detection, UV at 254 nm. (B) Column same as in (A) except column length, 217 cm; mobile phase, hexane with 0.12% methanol; linear velocity, 1.1 cm/sec. Peaks as in Fig. 1.

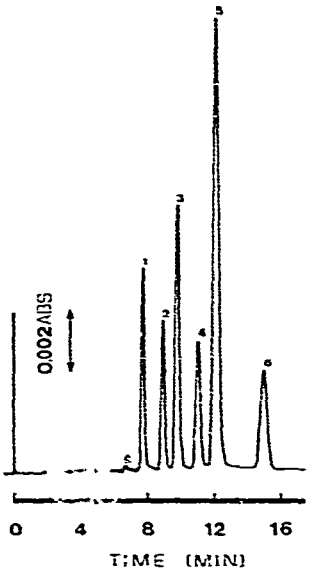

Fig. 6. Separation of model polycyclic aromatic hydrocarbons on a silica–octadecylsilane microcapillary column. Column, 232 cm × 40 μm I.D. packed with 10 μm silica gel modified to ODS; mobile phase, methanol–water (82:18); linear velocity, 0.6 cm/sec; inlet pressure, 150 kg/cm². Peaks: S = solvent; 1 = benzene; 2 = naphthalene; 3 = biphenyl; 4 = fluorene; 5 = anthracene; 6 = pyrene.

generally considered to be a good indication of the chromatographic quality of a column across a range of particle sizes[16]. The microcapillary of 75 μm I.D. and particle size 30 μm (curve 3) has been treated as the optimum one in previous reports[3–6]. The packed microcapillary of the present work (curve 2) shows a very similar profile. Although the initial h values of curves 1 and 2 (particle size 10 μm) are nearly the same, curve 2 is shifted generally towards a four times larger reduced velocity than curve 1. In the other words, the packed microcapillary of the present work is a considerable improvemenet on the previous (curve 1)[3]. At the same reduced velocity, curve 2 has a slightly larger h value than curve 3. But, for a given theoretical plate number, the capillary of curve 2 can be operated in an analysis time of *ca.* 20% of that of the microcapillary of curve 3.

Simple calculation of the theoretical plate numbers of 10 m and 40 m packed microcapillary columns in the present experiment gives 50,000 and 200,000 plates, respectively and the analysis times for a solute with $k' = 1$ are 33 and 133 min, respectively. These analysis times are reasonably acceptable for high-resolution LC.

Typical examples of applications are shown in Figs. 5 and 6. The theoretical plate number for N-ethylaniline ($k' = 0.56$) in Fig. 5A is almost 100,000. A fast analysis with a short microcapillary (*ca.* 2 m long) is shown in Figs. 5B and 6. Theoretical plate numbers for solutes in Figs. 5B and 6 are from 5000 to 8000. These chromatograms are very similar to those that would be obtained with a good ordinary column, *i.e.* a 2 m microcapillary column is the length that would correspond to a good ordinary column. So a long microcapillary, 2x m in length, would show x times larger plate numbers than an ordinary column. It should be emphasized that it is not difficult to make a microcapillary column as long as 40 m.

ACKNOWLEDGEMENT

This work was supported by a grant-in-aid for Scientific Research from the Ministry of Education of Japan (No. C56550533) and The Toyota Foundation (No. 81-1-252).

REFERENCES

1 C. E. Reese and R. P. W. Scott, *J. Chromatogr. Sci.*, 18 (1980) 479.
2 R. P. W. Scott and P. Kucera, *J. Chromatogr.*, 125 (1976) 251.
3 T. Tsuda and M. Novotny, *Anal. Chem.*, 50 (1978) 271.
4 Y. Hirata, M. Novotny, T. Tsuda and D. Ishii, *Anal. Chem.*, 51 (1979) 1807.
5 Y. Hirata and M. Novotny, *J. Chromatogr.*, 186 (1979) 521.
6 M. Novotny, *J. Chromatogr. Sci.*, 18 (1980) 473.
7 T. Tsuda, K. Hibi, T. Nakanishi, T. Takeuchi and D. Ishii, *J. Chromatogr.*, 158 (1978) 227.
8 T. Tsuda and G. Nakagawa, *J. Chromatogr.*, 199 (1980) 249.
9 T. Tsuda, K. Tsuboi and G. Nakagawa, *J. Chromatogr.*, 214 (1981) 283.
10 D. Ishii and T. Takeuchi, *J. Chromatogr. Sci.*, 18 (1980) 462.
11 J. H. Knox, *J. Chromatogr. Sci.*, 18 (1980) 453.
12 J. H. Knox and M. T. Gilbert, *J. Chromatogr.*, 186 (1979) 405.
13 G. Taylor, *Proc. Roy. Soc. London, Ser. A*, 219 (1953) 186.
14 R. K. Gilpin, J. A. Korpi and C. A. Janicki, *Anal. Chem.*, 46 (1974) 1314.
15 T. Tsuda and D. Ishii, *The 22nd Symposium on Liquid Chromatography, Kyoto, Japan, February 16, 1979.*
16 J. C. Giddings, *Dynamics of Chromatography*, Marcel Dekker, New York, 1965.