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OPEN-TUBULAR LIQUID CHROMATOGRAPHY WITH 5–10-μm I.D. COLUMNS*

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SUMMARY

The use of micro-bore capillary columns of I.D. 5–10 μm in liquid chromatography is discussed. Separations using 6–11-μm I.D. columns are described, and their h–v relationships are discussed in relation to theoretical values. An apparatus for in-column injection is described.

INTRODUCTION

Recent attention in the area of open-tubular liquid chromatography (LC) has been focused on the chromatographic behaviour of very micro-bore columns[1–5]. Theoretical studies of open-tubular columns in LC have also suggested that there are excellent prospects for capillary liquid chromatography if it is possible to reduce the inner diameter sufficiently and to solve the technical problems of detection and injection in LC capillary systems[6]. Research using an open-tubular capillary column of I.D. less than 30 μm has been carried out by Tijssen *et al.*[1], Krejčí *et al.*[2], Tsuda *et al.*[3], Jorgenson and Guthrie[4] and others. However, it is still necessary to make further developments in this area and to establish experimentally the abilities of micro-bore capillary LC.

In this paper, we discuss open-tubular capillary LC with 5–11-μm columns. Our calculations[3] suggest that this size of column may be the ultimate limit in capillary LC.

THEORETICAL

The volume flow-rate with a 10-μm capillary column with a linear flow-rate of 1 cm/sec is only 0.78 nl/sec. If the standard deviation of a peak is 2 sec, the detector volume must be less than 0.78 nl, because its volume should not exceed half of the volume standard deviation[6]. If the retention time and standard deviation of a peak

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are 600 sec and 2 sec, respectively, the theoretical plate number (N) is 90,000. When the peak is non-retained, this plate number is about one-sixth of the theoretical value calculated with the following parameters: diffusion coefficient (D_m), 10^{-5} cm^2/sec; linear flow-rate, 1 cm/sec; column, 6 m × 10 μm I.D.

A solute that stays in the tubing of a 5-μm capillary column and with a D_m assumed to be 10^{-5} cm^2/sec in the mobile phase reaches and leaves the capillary wall about 100 times per second, if we could calculate the movement of a solute using the equation $d^2 = 2D_m t$ (where d and t are the inner diameter of the capillary column and the time period over which diffusion occurs, respectively)[7].

As a capillary diameter of 5–10 μm is approximately the same as the particle size of silica gel used in conventional columns, the inner surface area per the column length of such diameter of the capillary column would be nearly equal to the surface area of one particle of silica gels, if the inner surface of the capillary column is similar in character to the surface of silica gel, and the radius of the capillary is assumed to be unity.

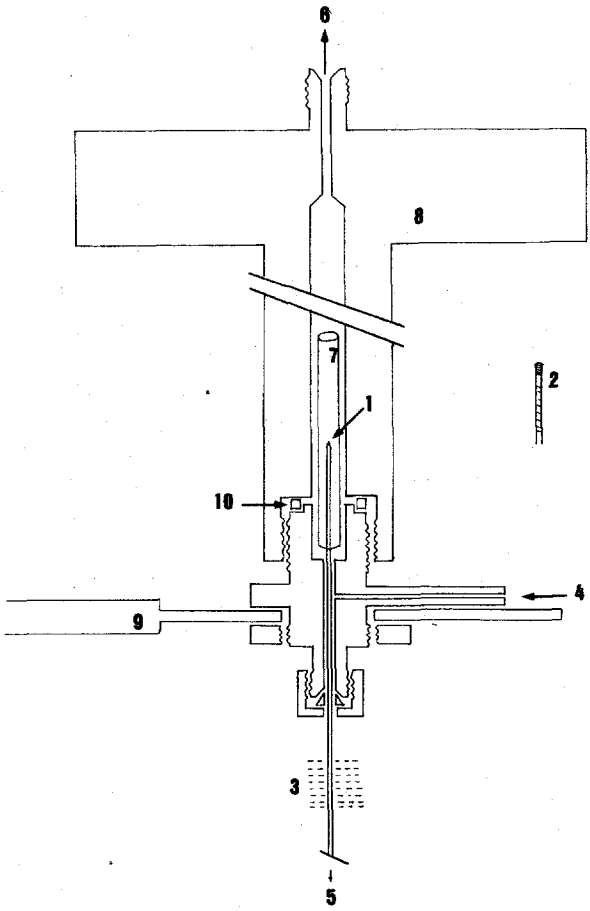

Fig. 1. Apparatus for in-column injection. 1 = Head of capillary column; 2 = PTFE tubing, one of the ends of which was sealed; 3 = heating zone; 4–6 = directions of effluent flow; 7 = guard tube; 8 = cap; 9 = support; 10 = O-ring, made by PTFE resin.

EXPERIMENTAL

Preparation of capillary columns

Treatment of a soda-lime glass capillary with 0.3 *N* sodium hydroxide solution at 50°C overnight, followed by washing, gave a modified inner surface that allowed it to be used as a column[3,8] either without further treatment or followed by a chemical bonding process[3]. Other procedures used in the preparation of capillary columns were similar to those in ref. 3.

Injection and detection

When we use capillary LC with very micro-bore columns, it is necessary to take appropriate precautions with the injection, detection and connections in order to prevent additional band broading in these components. Split[9] or in-column injection[3] was used.

A schematic design of the apparatus for in-column injection is shown in Fig. 1. The operating procedure was as follows. After removing the cap (8 in Fig. 1) and guard tube (7), a tube (2) containing the sample solution was attached on the head of the capillary column (1). Then component 3, of length 1.5 cm, was heated by a heater or a heat gun until the effluent in the capillary was converted into gas. After the heating had been stopped, the gas was condensed and, at the same time, the sample solution was led into the capillary head. With a 5-μm capillary column, the amount injected was 0.3 nl. Then the tube 2 was removed from the capillary head, and the head 1 was washed by the effluent solution. After 7 and 8 had been set, effluent was passed through to 6 for cleaning for a short time, then the effluent flow to 6 was stopped. The effluent was passed into the capillary column at an adequate inlet pressure. This apparatus could be operated up to 250 atm pressure.

An SPD-2AS UV detector (Shimadzu, Kyoto, Japan) was used. The UV cell and the connection between the cell and the outlet of the column were similar to those in ref. 3. Fused-silica capillary tubing (25 or 50 μm I.D.) was connected directly to the outlet of the column with a small piece of PTFE tubing. The lengths of the connection and the cell were 1.5 and 0.7 mm, respectively. The cell volume, which was dependent on the inner diameter of the fused-silica capillary used, ranged from 1.4 to 0.34 nl.

RESULTS AND DISCUSSION

Capillary liquid chromatography with ca. 5-μm I.D. columns

Although split injection was applicable to capillary LC with 10 μm I.D. columns, it was necessary to used the in-column injection method for LC with around 5-μm I.D. columns.

A chromatogram using a capillary column of 220 cm × 6 μm I.D. is shown in Fig. 2. This chromatogram may be the first obtained with capillary column of inner diameter less than 10 μm. The theoretical plate number of peak 6 in Fig. 2 is about 9000.

Capillary liquid chromatography with ca. 10-μm I.D. columns

Two chromatograms obtained by using *ca.* 10-μm I.D. capillary columns are

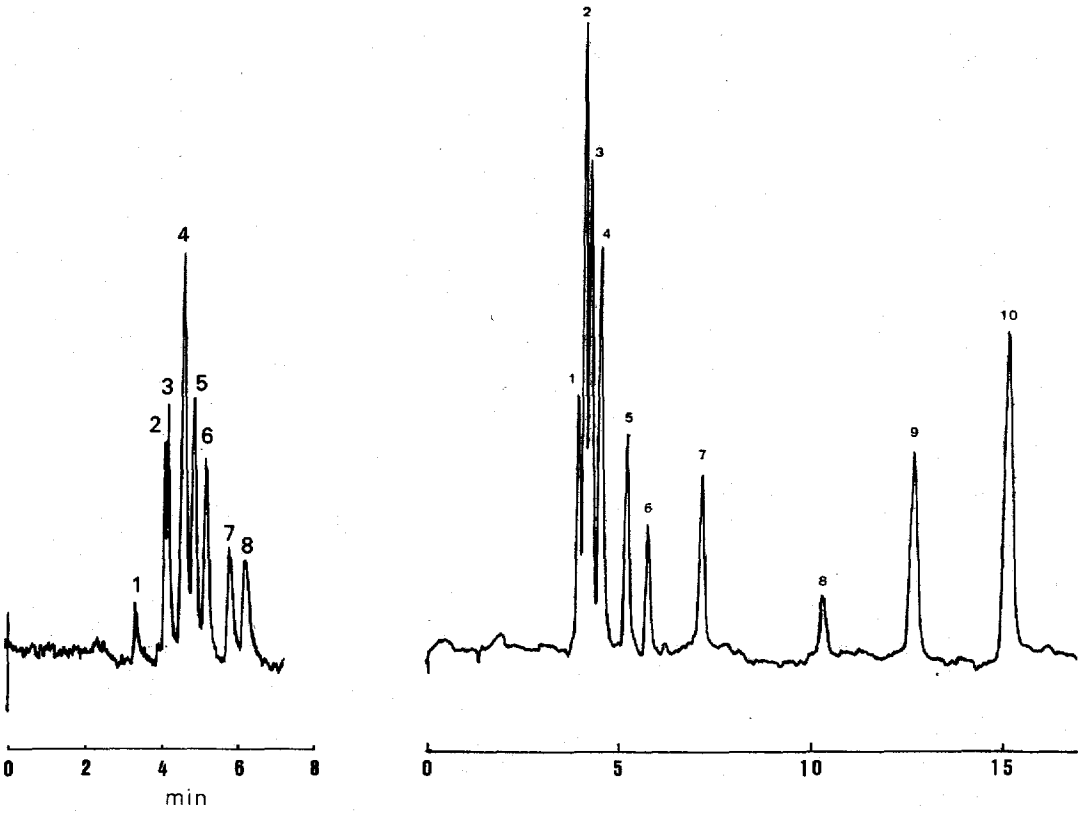

Fig. 2. Chromatogram obtained by using a 6-μm I.D. column of length 220 cm. Mobile phase: *n*-hexane containing acetonitrile (0.7%), methanol (0.7%), dichloromethane (0.3%) and water (0.01%). Sample: (1) *o*-, (7) *m*- and (8) *p*-chloroaniline; (2) 3-chloro-*p*- and (4) 4-chloro-*o*- and 6-chloro-*o*-toluidine; (3) *o*-, (5) *m*- and (6) *p*-toluidine. Inlet pressure: 110 atm. UV detection at 235 nm.

Fig. 3. Separation of aromatic amines. γ-Aminopropylsilane column (330 cm × 10 μm I.D.). Column temperature: 11°C. Mobile phase, upper layer of the hexane–acetonitrile–dimethyl sulphoxide (87:10:10). Linear flow velocity: 1.4 cm/sec. Sample: (1) N,N-dimethyl-, (2) N-butyl-, (3) N-propyl-, (4) N-ethyl- and (5) N-methylaniline; (6) N-phenyl-α- and (7) N-phenyl-β-naphthylamine; (8) aniline; (9) α- and (10) β-naphthylamine.

shown in Figs. 3 and 4. The theoretical plate number of α-naphthylamine (9 in Fig. 3) and dimethyl phthalate (5 in Fig. 4) are 38,000 and 50,000, respectively, and the numbers of plates per second are 50–60.

Under the experimental conditions used in Fig. 4, the relationship between height equivalent to a theoretical plate (H, μm) and linear velocity (v, cm/sec) was

$$H = 34.7v + 28.8 \quad (1)$$

At zero velocity, the value of H was 28.8 μm, which means that the value of the reduced plate height is about 3.

The relationship between reduced plate height (h) and reduced velocity (v) is

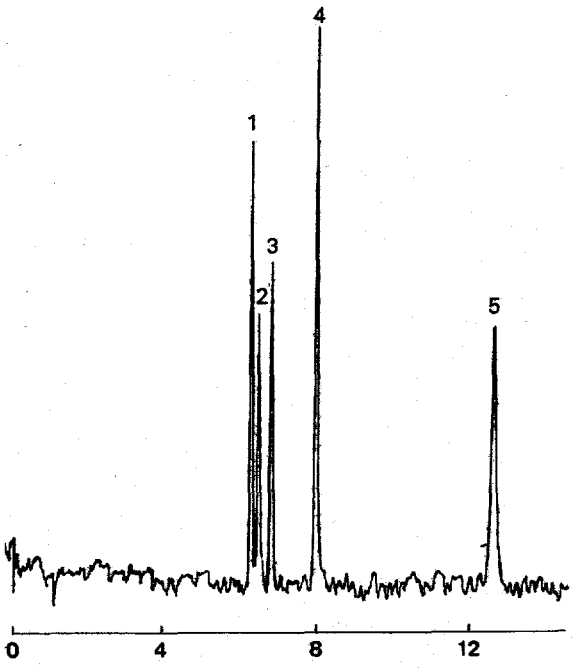

Fig. 4. Separation of phthalates. γ-Aminopropylsilane column (830 cm × 11 μm I.D.). Mobile phase as in Fig. 3. Linear velocity: 2.3 cm/sec. Inlet pressure: 190 atm. UV detection at 254 nm. Sample: (1) dioctyl, (2) dibutyl, (3) dipropyl, (4) diethyl and (5) dimethylphthalate.

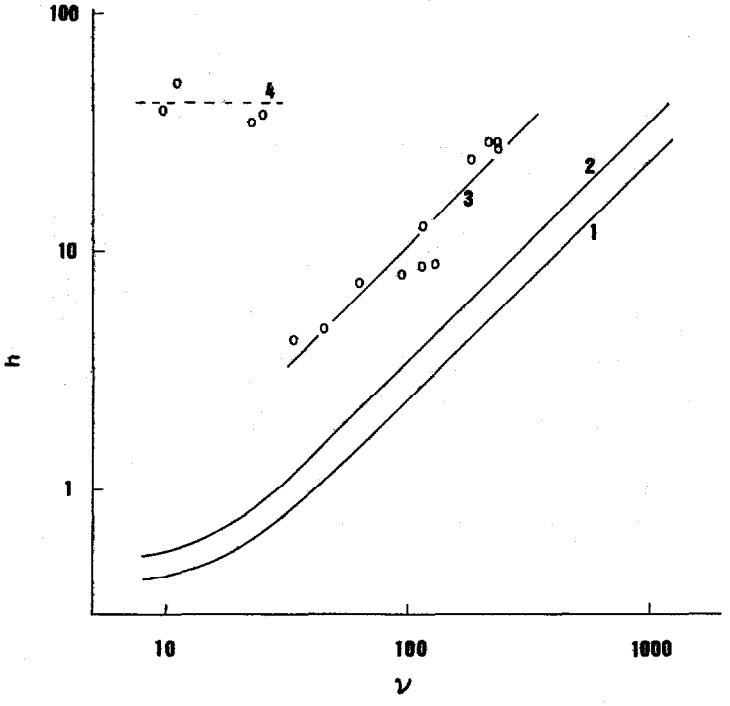

Fig. 5. Relationship between reduced plate height and reduced flow velocity. (1) and (2) are theoretical lines for $k' = 0.30$ and 0.60, respectively. (3) Column, I.D. 11 μm; solute diethyl phthalate ($k' \approx 0.3$); $D_m = 2.26 \cdot 10^{-5}$ cm²/sec; other conditions as in Fig. 4. (4) Column, I.D. 6 μm; solute, *p*-toluidine ($k' \approx 0.6$); $D_m = 3.35 \cdot 10^{-5}$ cm²/sec; other conditions as in Fig. 3.

shown in Fig. 5. Two theoretical lines are obtained by using the following equation (k' = capacity factor):

$$h = 2v^{-1} + (1 + 6k' + 11k'^2)(1 + k')^{-2}v \quad (2)$$

For the determination of diffusion coefficients, the Wilke–Chang equation[7,10] was used. The experimental line for an 11-μm I.D. column (3 in Fig. 5) gives almost a 4-fold larger value of h than the theoretical line, but the line for a 6-μm I.D. column gives about a 60-fold larger value of h than the theoretically predicted values. Although the experimental values differ from the theoretical values, the present experimental line for an 11-μm I.D. column is nearly the same as that obtained using a 14-μm I.D. column for a non-retained solute and better than that obtained using a 9-μm I.D. column for a non-retained solute given by Krejčí *et al.*[2]. The present h–v relationship for an 11-μm I.D. column is nearly the same as that for a non-related solute using a 10-μm I.D. column given by Tijssen *et al.*[1].

From the present experimental results and those obtained by Tijssen *et al.*[1], microcapillary LC with a *ca.* 10-μm I.D. column shows good separative abilities in accordance with theoretical predictions.

Although the h value obtained using a 6-μm I.D. column is not good compared with the theoretical value, we obtained a separation using this column. Our results indicate that on-column injection is superior to split injection for very micro-bore columns. This technique might be useful in the further development of very micro-bore capillary LC, such as open-tubular capillary LC with columns of I.D. less than 5 μm.

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