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OPEN-TUBULAR MICROCAPILLARY LIQUID CHROMATOGRAPHY WITH 20-μm I.D. COLUMNS

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SUMMARY

Capillary columns of ethylene cyanohydrin, octadecylsilane and silica of I.D. 20 μm were prepared. For pre-treatment of a soda-lime glass capillary of I.D. 20 μm with sodium hydroxide solution a concentration of 0.5–0.7 *N* was optimal. A detection system with a small inner volume, such as 0.019 μl, was made. As extra-column effects increase with the fourth power of the ratio of the capillary column radius to the connections and cell radius, the latter values should be kept as small as possible.

INTRODUCTION

Although open-tubular microcapillary liquid chromatography is in the development stage[1–11], there is now increasing interest for its future ability. The practical inner diameters of capillary columns have decreased from 60–50 μm in 1978[2,3] to 40–30 μm in 1980[9,11]. A theoretical calculation of capillary column efficiency predicts that a smaller inner diameter column gives a higher theoretical plate number per unit time with reduced injection and detection volumes[8].

If the optimal conditions in open-tubular capillary gas chromatography are calculated for a linear velocity of 10 cm/sec with a 0.25-mm I.D. column for a solute with an interdiffusion coefficient assumed to be 10^{-1} cm^2/sec, these parameters should be reduced in open-tubular microcapillary liquid chromatography owing to the small interdiffusion coefficient, *e.g.*, 10^{-5} cm^2/sec. For example, if the flow velocity is set at 1 cm/sec, the I.D. of the capillary column in liquid chromatography should be about 8 μm.

We consider this size of column currently to be the final limit in capillary liquid chromatography. Before treating this very narrow capillary column, a 20-μm I.D. capillary column is discussed in this paper with respect to the development of a technique that would reduce extra-column effects and could be used for preparing the column. The cross-sectional area of a 20-μm I.D. column is about half that of a 30-μm I.D. column, and four times larger of that of a 10-μm I.D. column.

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EXPERIMENTAL

Preparation of capillary columns

Treatment of a soda-lime glass capillary with an alkaline solution gave a modified inner surface that worked as an adsorbent column either without further treatment[6] or followed by a stationary phase coating process[3,11]. The treatment process with an alkaline solution was as follows: sodium hydroxide solution was placed in the capillary tubing and kept at 50°C for 18 h, then the capillary was washed with methanol, acetonitrile, dichloromethane and finally with *n*-hexane.

For filling, the alkaline solution was forced into capillary tubing by nitrogen gas at 15–70 atm. Under a pressure of 20 atm, connection of PTFE tubing (0.1 mm I.D.) with glass capillary or stainless-steel tubing (0.1 and 0.3 mm O.D.) could be used. However, above 20 atm one end of the capillary was kept in a 1/4-in. stainless-steel tube and the capillary was set in a polyimide ferrule (1/16 in.) using a 1/4–1/16 in. reducing union. PTFE tubing was attached to the capillary head and another PTFE tube, which was filled with the alkaline solution, was connected to the first PTFE tube by stainless-steel tubing (0.6 mm O.D.). The capillary head and alkaline solution were kept in the pressurized atmosphere, so there are no leakage problems.

To determine the amount of silica gel that was formed on the inner capillary tubing, the capacity factor (k') of β-naphthylamine was examined using *n*-hexane as the mobile phase. The results are shown in Fig. 1. The maximal effect was given by using *ca.* 0.7 *N* sodium hydroxide solution for 20-μm I.D. tubing, but 1 *N* solution for 50-μm I.D. tubing[6]. The k' value of β-naphthylamine for 20-μm I.D. tubing is about 10 times greater than that of same sample for 50-μm I.D. tubing under same treatment conditions. It is suggested that the phase ratio (mobile phase to adsorbent layer)

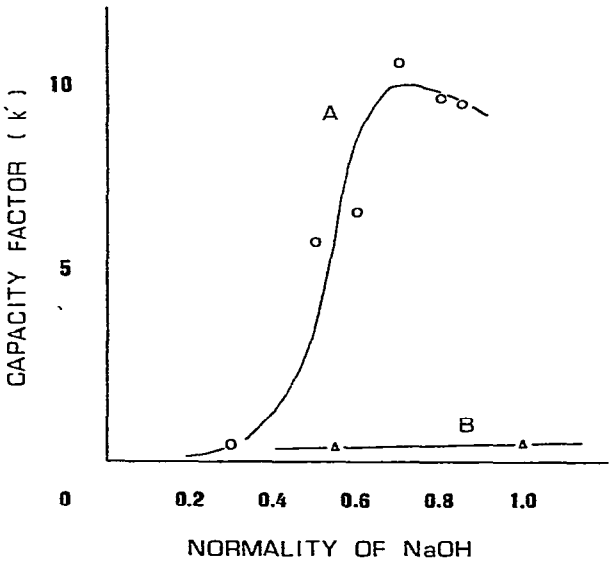

Fig. 1. Effect of concentration of sodium hydroxide solution on k' value of β-naphthylamine. The glass capillary was treated with sodium hydroxide solution of different concentrations for 18 h at 50°C. A, 20-μm I.D. capillary column; B, 50-μm I.D. capillary column.

might be changed considerably. A concentration of sodium hydroxide solution above 1 *N* was not suitable for 20-μm I.D. capillary tubing, because the capillary often became clogged.

Ethylene cyanohydrin stationary phase

Ethylene cyanohydrin was coated by the modified dynamic coating method as follows[5]: four coils of glass capillary that had been pre-treated with alkaline solution were filled with dichloromethane, and a plug of 20% ethylene cyanohydrin–dichloromethane solution (about 10 μl) was forced into the capillary at a rate of about 3 cm/sec by using nitrogen at 70 atm. After coating, the capillary was kept under a flow of nitrogen, and then was placed in at oven with the temperature programmed at 0.25°C/min to 60°C and kept at 60°C overnight under a flow of nitrogen.

Octadecylsilane (ODS) stationary phase

After the capillary had been filled with xylene, about 50 μl of octadecyltrieth-oxysilane solution was passed through under a pressure of 18 atm (semi-static method)[7]. The concentration of ODS in xylene was examined in the range from 0.2 to 1.0% (v/v) and the reaction temperature from 65 to 110°C. The optimal conditions were found to be 0.5–1.0% and 95°C, respectively. Then nitrogen gas was passed through the capillary for 2–3 h with the temperature programmed at 0.5°C/min from 60 to 110°C, followed by moist nitrogen gas overnight at 130°C. Finally, the capillary was dried with nitrogen at 130°C for 2–3 h. The column was conditioned with dichloromethane, acetonitrile and methanol before analytical use. The use of octadecyltrieth-oxysilane instead of octadecyltrichlorosilane decreased the tendency for clogging to occur.

"In-column" injection

Split injection[1,11] and a new "in-column" injection technique were used. The latter method was as follows. The capillary column head was set in a vertical position using a Swagelock union and polyimide ferrule. The mobile phase in the capillary column head part (length about 5 cm) was removed with a hair-dryer. Then, using a microburner, the section up to 10 cm from the capillary column head was heated gently for several seconds in order to expand or partially vaporize the eluent in the capillary, until the level of the eluent fell nearly to the capillary head, then the microburner was removed. Immediately afterwards, the eluent started to rise owing to condensation of the vapour, and the capillary head was dipped in the sample solution so that the sample solution was sucked into the capillary column in a few seconds. After measuring the length of sample sucked in (an air bubble was used as the marker), the capillary column head was washed and connected to the pump, and a chromatographic run was started.

This new injection method is very simple and is completely free from an extra-column volume. If the capillary is kept in an oven, the microburner and dryer might be replaced by simply changing the oven temperature.

UV detection systems

Two different UV detection systems, type A and B, were made. The UV quartz cell in system A was coupled directly with the capillary column outlet using PTFE

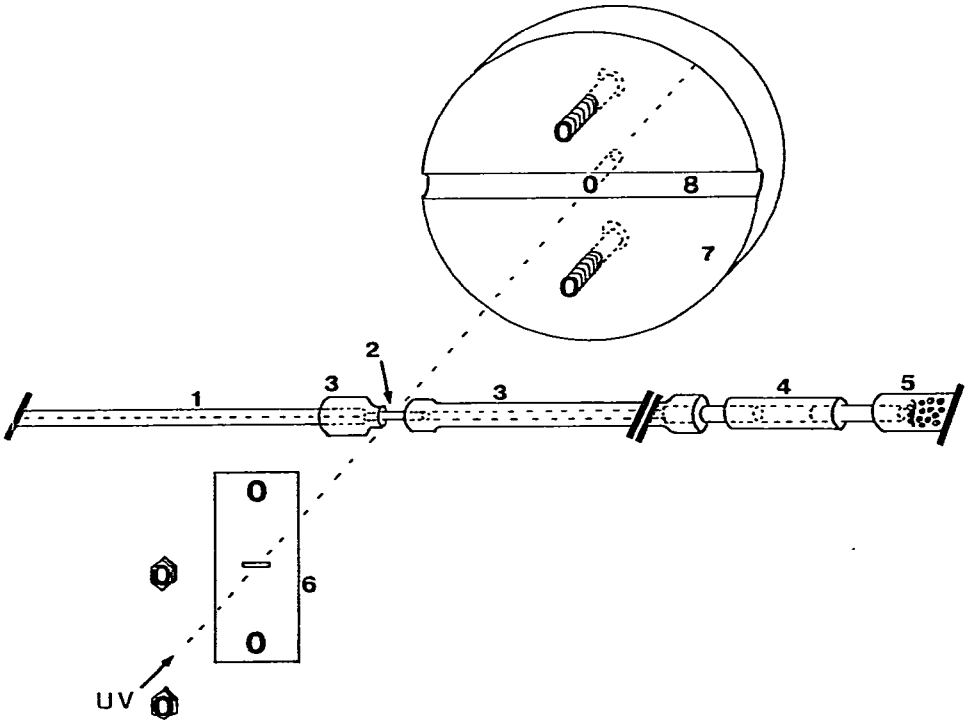

Fig. 2. Schematic diagram of UV detection system A. 1 = Capillary column; 2 = quartz cell, I.D. 0.1 mm, O.D. 0.3 mm; 3 = PTFE tubing, I.D. 0.1 mm, O.D. 1.6 mm; 4 = PTFE tubing, I.D. 0.5 mm, O.D. 1.0 mm; 5 = back-resistance, I.D. 0.5 mm × 1.5 mm O.D. × 20 mm length, PTFE tubing packed with 10-μm silica gel; 6 = slit; 7 = holder made of rubber; 8 = groove for quartz cell and its connections.

tubing, as shown in Fig. 2. A column outlet section (length 15 mm) and a quartz cell (I.D. 0.1 mm) were fixed by using adhesive on the cell holder, which was made of rubber. The length between the column outlet and the centre of the cell was 2 mm, so the volume of this part was 0.019 μl. System B was similar to system A except that tubing of different radius and extra connections were used for easy handling[3]. The column outlet was interfaced with stainless-steel tubing (I.D. 0.11 mm and length 7 mm) then PTFE tubing (I.D. 0.07 mm and length 20 mm). The last component was a quartz cell (I.D. 0.12 mm and length 2 mm) from the inlet to the centre of the UV light spot. The total volume of system B was 0.17 μl.

The other chromatographic operations were virtually the same as those used previously[11].

RESULTS AND DISCUSSION

Detection system B could be operated with a low noise level similar to that with the usual detectors, but system A showed much higher noise than system B (as shown in Figs. 4 and 5). One of the reasons might be vibration of the quartz cell, although this cell and the outlet part of the column were fixed tightly by adhesive.

Fig. 3 shows the differences in the chromatograms due to the different post-column systems. Chromatogram A was obtained by using a 20-μm I.D. capillary column and detection system A, and chromatogram B was obtained by using the same capillary column and detection system B. The k' values of the peaks are nearly zero and 0.05, respectively. The heights equivalent to a theoretical plate for the peaks in chromatogram A are 3–4 times larger than those in chromatogram B. The difference in the retention time of the first peak between chromatograms A and B is due to the extra time required for travelling between the column outlet and the UV cell. The extra-column effect, as shown in Fig. 3, is considerable for the peak with a small k' value, but it gradually decreases with increasing of k', as predicted by eqn. 1 below.

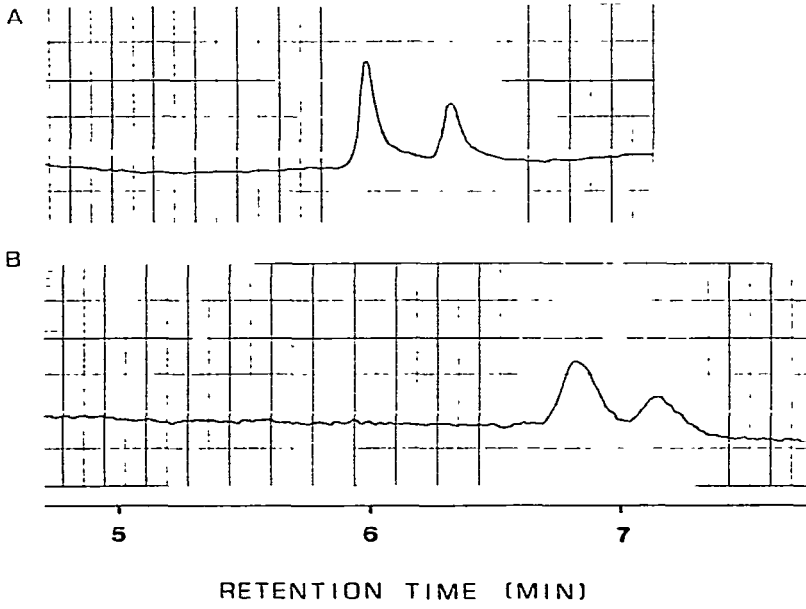

Fig. 3. Extra-column effect on chromatogram due to different detection systems. Chromatograms A and B were obtained by using detection system A and B, respectively, under same experimental conditions. Capillary column, I.D. 20 μm and length 5.5 m; mobile phase, *n*-hexane. First peak, N,N-diethylaniline; second peak, N-phenyl-α-naphthylamine.

Peak broadenings due to column and extra-column effects, which includes the connection and detection parts, are estimated by the factors f_c and f_{ext}, respectively, which were defined previously[11]. The ratio f_{ext}/f_c is

$$f_{ext}/f_c = [\Sigma L_{con,i}\,(r_{con,i}/r_c)^4 + L_d\,(r_d/r_c)^4]\,R_c^2 L_c^{-1}\,(6R_c^2 - 16R_c + 11)^{-1} \quad (1)$$

where L, r and R are the length, radius and the ratio of zone velocity to the velocity of the mobile phase, respectively. The suffixes con, d and c represent connection, detector and column part, respectively; i means a local part. As the radius term in eqn. 1 is raised to the fourth power, r_{con} and r_d should be kept as small as possible relative to r_c. The calculated values of f_{ext}/f_c for detection systems A and B are given in Table I.

TABLE I

CALCULATION OF EFFECT OF EXTERNAL COLUMN VOLUME* ON COLUMN EFFICIENCY

Column		*k'*	f_{ext}/f_c	
I.D. (μm)	*Length (m)*		*Detection system A*	*Detection system B*
20	30	0	0.042	0.40
		1	0.0023	0.022
		2	$2.3 \cdot 10^{-4}$	0.0070
	5	0	0.25	2.39
		0.5	0.37	0.36
		1	0.014	0.13
		2	0.0044	0.042
		3	0.0021	0.020
10	5	0	3.9	38
		1	0.22	2.1
		2	0.070	0.67
		3	0.034	0.33

* For the design of this part, see text.

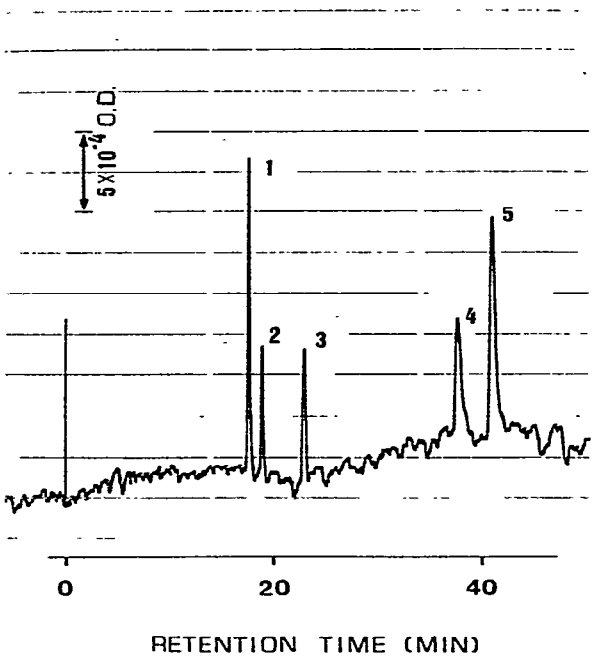

Fig. 4. Separation of aromatic amines on a silica column modified with hexylamine using detection system A. Column, I.D. 22 μm and length 560 cm; mobile phase, 0.05% hexylamine–*n*-hexane; linear velocity, 7 mm/sec. Samples: 1 = N,N-diethylaniline; 2 = N-phenyl-α-naphthylamine; 3 = N-phenyl-β-naphthylamine; 4 = α-naphthylamine; 5 = β-naphthylamine.

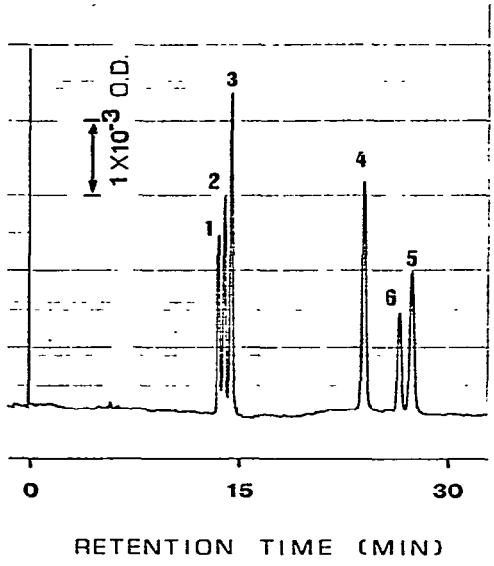

Fig. 5. Separation of aromatic amines on an ethylene cyanohydrin column using detection system B. Column, I.D. 23 μm and length 10.5 m; mobile phase, *n*-hexane saturated with ethylene cyanohydrin; linear velocity, 13 mm/sec. Samples: 1–5 as in Fig. 4; 6 = aniline.

Detection system A could be used even for a column of I.D. 10 μm and length 5 m for a sample with a k' value greater than 1. Detection system B for the column of I.D. 20 μm and length 5 m can only be used when k' is greater than 1 in the respect of extra-column effect.

Chromatograms obtained with the 20-μm I.D. capillary column are shown in Figs. 4 and 5 for detection systems A and B, respectively. The height equivalent to a theoretical plate (H) at a linear velocity of 1 mm/sec was calculated from the assumption for comparison of column efficiency that there is a first-order linear relationship with zero intercept between H and linear velocity in capillary liquid chromatography[3,5,6,11]. Calculated H values at a linear velocity of 1 mm/sec for N-phenyl-α-naphthylamine (k' = 0.03), N-phenyl-β-naphthylamine (k' = 0.07), α-naphthylamine (k' = 0.77), aniline (k' = 0.96) and β-naphthylamine (k' = 1.14) with an ethylene cyanohydrin column, shown in Fig. 5, were 0.029, 0.026, 0.016, 0.018 and 0.023 mm, respectively, at room temperature using *n*-hexane saturated with ethylene cyanohydrin as the mobile phase. H values at a linear velocity of 1 mm/sec for naphthalene (k' = 0.22), biphenyl (k' = 0.38), anthracene (k' = 0.51), phenanthrene (k' = 0.88) and pyrene (k' = 1.39) with an ODS column (I.D. 23 μm and length 4.4 m) were 0.25, 0.28, 0.23, 0.21 and 0.19 mm, respectively, at room temperature using water–acetonitrile (1:1) as the mobile phase. An ethylene cyanohydrin column gave almost one-tenth the H values for aromatic amines compared with those obtained with an ODS column for fused aromatics. Although the viscosity of water–acetonitrile solution is higher than that of *n*-hexane, the preparation procedure for the ODS column should still be improved. In Fig. 4 the mobile phase contained 0.05% of

hexylamine; without this addition the column worked as an adsorbent column, but with addition of hexylamine it worked as if it had a physically adsorbed layer of hexylamine, *i.e.*, as a liquid–liquid chromatographic column.

The chromatograms in Figs. 4 and 5 do not show any substantial broading of the later peaks. This suggests that narrow capillaries would have a high resolving ability, as predicted by theoretical considerations[1,8]. Capillary liquid chromatography using a column of I.D. 10 μm is now under investigation.

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