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HIGH-PERFORMANCE MICRO PACKED FLEXIBLE COLUMNS IN LIQUID CHROMATOGRAPHY

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SUMMARY

Columns of flexible fused-silica tubing packed with commercially available materials have been prepared. The performance of these columns was examined with acetonitrile–water as the mobile phase and polynuclear aromatic hydrocarbons as the solutes. A 10 cm × 0.25 mm I.D. column yielded about 7000 plates (plate height 14 μm), a performance comparable to that of conventional high-performance liquid chromatography. The effects of column length and injection volume on the column efficiency are discussed.

INTRODUCTION

The use of microbore packed columns has been examined by a number of workers: Ishii *et al.*^{1–7} have produced 3–20 cm × 0.25–0.5 mm I.D. columns; Novotny and co-workers^{8–10} have investigated packed microcapillary columns, 10–60 m × 0.05–0.2 mm I.D. and Scott and co-workers^{11–16} have examined 10–100 cm × 0.5–1 mm I.D. columns. Each column possesses inherent properties and characteristics.

Lower volumetric flow-rates can be selected in small-bore column liquid chromatography (LC) than in conventional high-performance liquid chromatography (HPLC), which results in less consumption of the mobile phase and the facility of direct combination of LC and mass spectrometry.

Recently¹⁷, we have investigated the effect of column material on column performance using ultra-micro columns (3–10 cm × 0.12–0.15 mm I.D.) and found that Pyrex glass columns possessed the highest efficiency. However, such columns are fragile and difficult to handle. Thus, mechanically strong materials which have a glassy surface are desirable. Fortunately, flexible fused-silica tubings are commercially available which are mechanically strong and have inert surfaces, and can therefore be employed as column materials in micro-HPLC.

Flexible fused-silica capillary columns have been employed in gas chromatography for some years. On the other hand, few studies using fused-silica columns have been reported in capillary LC¹⁸. The present article describes the application of micro packed flexible columns to LC and their chromatographic performances.

TABLE I
 DIMENSIONS AND EFFICIENCIES OF COLUMNS EXAMINED
 Packing material: silica ODS SC-01. Mobile phase: acetonitrile-water. N = Theoretical plate number.

Column I.D. (mm)	Length Material (cm)	Mobile phase composition (v/v)	Flow- rate (μ /min)	Temp. ($^{\circ}$ C)	Efficiency		Pyrene		HETP					
					Biphenyl k'	N	Biphenyl k'	N	Biphenyl (μ m)	N	Pyrene (μ m)	N		
0.25	3.0	Fused silica	1.7	23	3.8	1200	3.8	1200	4000	25	10.7	1500	4900	20
0.25	5.0	Fused silica	1.7	25	2.8	2600	2.8	2600	5200	19	7.1	2800	5600	18
0.25	10	Fused silica	1.7	22	2.1	6900	2.1	6900	6900	14	7.1	7400	7400	13
0.25	30	Fused silica	1.4	27	-	17,100	-	17,100	5700	18	-	19,500	6100	15
0.25	80	Fused silica	0.35	26	1.5	26,300	1.5	26,300	3300	30	3.5	23,700	3000	34
0.12	10.6	Pyrex glass	0.42	27	2.1	2300	2.1	2300	2200	46	4.8	3100	3000	34
0.50	9.8	PTFE	5.6	23	2.6	2100	2.6	2100	2100	47	6.2	2700	2700	37

EXPERIMENTAL

Apparatus

The liquid chromatograph was assembled from a micro feeder (Azumadenki Kogyo, Tokyo, Japan) and a gas-tight syringe (100 or 250 μl) as pump, a micro packed flexible column, UV spectrophotometer UVIDEC-100 with a modified flow cell (Japan Spectroscopic, Hachioji-shi, Japan) and a recorder. The flow cell comprised a quartz tube (0.19 mm I.D., 0.45 mm O.D.) connected to the exit of the column through PTFE tubing (0.25 mm I.D., 2 mm O.D.). Fused-silica tubing (75 \times 0.071 mm I.D.; Scientific Glass Engineering, Ringwood, Australia), PTFE tubing (0.25 mm I.D., 2 mm O.D.) and stainless-steel tubing (5 \times 0.13 mm I.D.) were employed as connecting tubing between the flow cell and the column. The connection volume between the flow cell and the column was around 0.4 μl . Samples were injected manually, as reported previously¹.

Column preparation

The packing technique employed was almost the same as in earlier studies^{1,17}. Fused-silica tubing (0.25 mm I.D., 0.36 mm O.D.; Gasukuro Kogyo, Tokyo, Japan) was usually employed as column material. PTFE tubing (0.25 mm I.D., 2 mm O.D.) was connected to both ends of a fused-silica tube and quartz wool was inserted in one end. The packing material, which had been dispersed in acetonitrile by ultrasonic vibration, was poured into the PTFE tubing and then passed into the silica tubing. After packing, the open end was filled with quartz wool to prevent leakage of packing from the column. Less than 1 mm in length of quartz wool should be used, otherwise band broadening of solutes in these parts cannot be neglected.

RESULTS AND DISCUSSION

The column performance was estimated by using silica ODS SC-01 (particle diameter 5 μm , Japan Spectroscopic) as the packing, acetonitrile-water as the mobile phase and polynuclear aromatic hydrocarbons as solutes. The capacity factor, k' , was calculated from

$$k' = (V_t^* - V_0^*)/V_0^* \quad (1)$$

where V_0 is the interstitial volume, V_t the retention volume of a solute, and V_0^* , V_t^* are corrected values, calculated by subtracting the connection volume from V_0 and V_t , respectively.

The effect of column length on the efficiency of flexible fused columns was examined (Table I). A 10-cm column gave the lowest height equivalent to a theoretical plate (HETP), 13–14 μm , which corresponds to 2.6–2.8 d_p (d_p is the particle diameter of the packing material). Columns of 5–30 cm in length had 5200–7400 theoretical plates per 10 cm, which compares favourably with conventional HPLC columns. On the other hand, 3- and 80-cm columns gave slightly lower efficiencies in terms of HETP, which may be caused by the extra-column effect in the former and the non-uniformity of the packing in the latter.

Table I also shows the performance of a PTFE micro column and a Pyrex glass ultra-micro column. The efficiencies of these columns were lower than those of 3–30

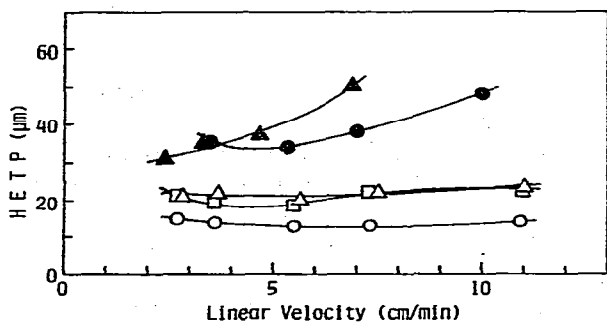


Fig. 1. Dependence of HETP on linear velocity. Columns: Δ , 3.0 cm \times 0.25 mm I.D., fused silica; \square , 5.0 cm \times 0.25 mm I.D., fused silica; \circ , 10 cm \times 0.25 mm I.D., fused silica; \blacktriangle , 9.8 cm \times 0.50 mm I.D., PTFE micro; \bullet , 10.6 cm \times 0.12 mm I.D., Pyrex glass ultra-micro. Mobile phase and k' values of pyrene as in Table I.

cm fused-silica columns. This result seems to be based on the differences between the inner surfaces of these tubings. Fused-silica tubing may possess smooth and inert surfaces.

Fig. 1 shows the dependence of HETP on the linear velocity of the mobile phase. It is seen that the dependence for fused-silica columns is much less than that for a PTFE micro column or a Pyrex glass ultra-micro column. This result seems to be due to the differences in smoothness and inertness of the inner surfaces of each column material, which may affect the packing near the column wall.

Fig. 2 shows curves of HETP *versus* linear velocity for a 10-cm fused-silica column. Very similar curves are obtained for biphenyl ($k' = 2.8$) and pyrene ($k' = 7.1$). Slightly larger HETP values are observed for benzene ($k' = 1.1$). The optimum flow-rate is the region between 5.5 and 7.3 cm/min (corresponding to 1.7–2.2 $\mu\text{l}/\text{min}$). However, for a 3-cm fused-silica column, some band broadening in the extra-column parts was observed for solutes having $k' < 3$.

Although the extra-column effect could be neglected for a 80-cm fused-silica column, a satisfactory column efficiency could not be obtained. This is ascribed to the non-uniform packing and the failure to operate at optimum flow, owing to the large

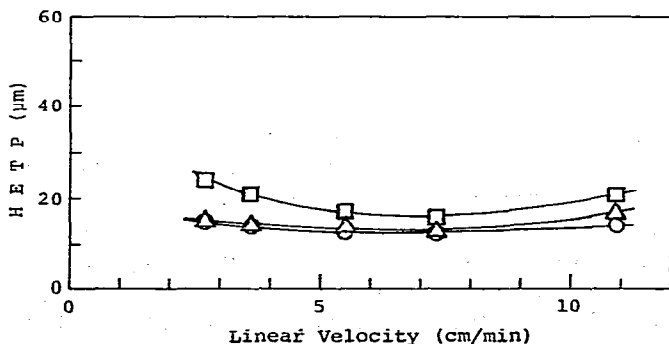


Fig. 2. Curves of HETP *versus* linear velocity for a 10 cm \times 0.25 mm I.D., fused-silica column. Mobile phase: acetonitrile–water (70:30). Column temperature: 22°C. Samples and k' values: \square , benzene ($k' = 1.1$); Δ , biphenyl ($k' = 2.8$); \circ , pyrene ($k' = 7.1$). Flow-rate, 10 cm/min = 3.06 $\mu\text{l}/\text{min}$.

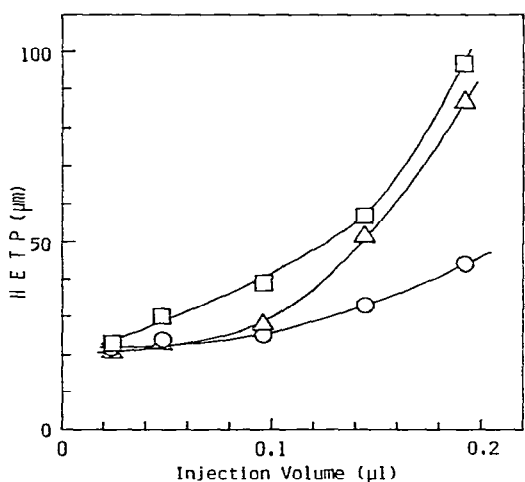


Fig. 3. The influence of sample volume on column efficiency. Column: 9.8 cm \times 0.25 mm I.D. Mobile phase: acetonitrile-water (70:30); flow-rate 2.2 μ l/min. Samples and k' values: \square , benzene ($k' = 1.2$); Δ , biphenyl ($k' = 2.9$); \circ , pyrene ($k' = 7.3$).

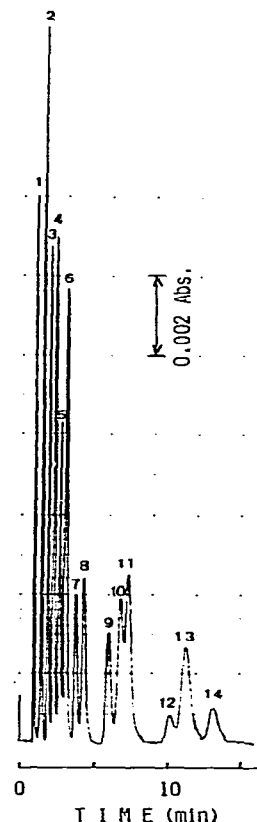


Fig. 4. Separation of polynuclear aromatic hydrocarbons on a 3 cm \times 0.25 mm column. Mobile phase: acetonitrile-water (60:40); flow-rate 3.3 μ l/min. Samples: 1 = 1.0% benzene; 2 = 0.097% naphthalene; 3 = 0.019% biphenyl; 4 = 0.022% fluorene; 5 = 0.0076% phenanthrene; 6 = 0.0058% anthracene; 7 = 0.017% fluoranthene; 8 = 0.020% pyrene; 9 = 0.020% *p*-terphenyl; 10 = 0.0074% chrysene; 11 = 0.0061% 9-phenylanthracene; 12 = 0.0025% perylene; 13 = 0.011% 1,3,5-triphenylbenzene; 14 = 0.0058% 3,4-benzopyrene. Injection volume: 0.02 μ l. Temperature: 23°C. Detection wavelength: 254 nm.

pressure drop across the column. Thus, 10–30 cm columns were preferred under our instrumental and operating conditions.

The influence of sample volume on the efficiency of a 9.8 cm \times 0.25 mm I.D. column is illustrated in Fig. 3. For solutes having $k' > 2$, no unfavourable effects were observed in the case of injections of less than 0.05 μ l, while for solutes having $k' < 2$, a slight deterioration of the column efficiency was observed in the case of the injection of 0.05 μ l. In the last case, the injection volume should be as small as possible. Small volumes of sample could easily be injected manually by the stop flow method. However, for routine or accurate quantitative analysis, a loop or a valve injector should be adopted. In addition, *ca.* 0.1 μ l of sample solution could be injected onto a 30-cm column without causing any unfavourable effects.

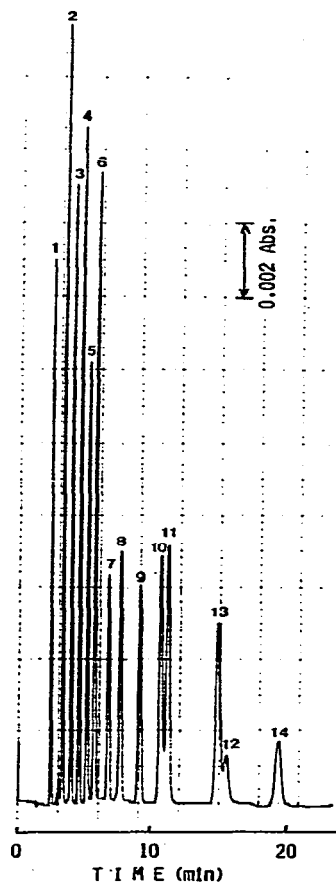
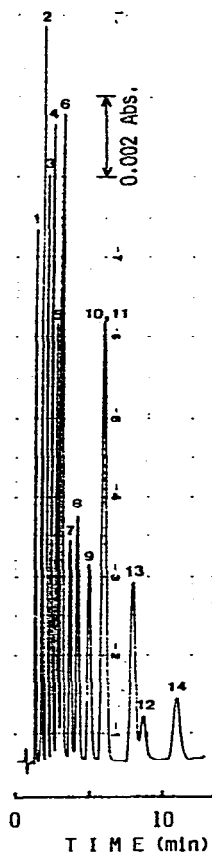


Fig. 5. Separation of polynuclear aromatic hydrocarbons on a 5 cm \times 0.25 mm I.D. column. Mobile phase: acetonitrile-water (70:30). Other operating conditions as in Fig. 4.

Fig. 6. Separation of polynuclear aromatic hydrocarbons on a 10 cm \times 0.25 mm I.D. Mobile phase: acetonitrile-water (70:30). Injection volume: 0.05 μ l. Temperature: 24°C. Other operating conditions as in Fig. 4.

Figs. 4-8 show the separations of polynuclear aromatic hydrocarbons obtained with several micro flexible columns packed with silica ODS SC-01. The 10-cm and 30-cm columns gave high resolutions, and even shorter columns, 3-cm and 5-cm, also gave reasonable separation. However, an 80-cm column gave slightly poorer results.

CONCLUSION

Flexible fused-silica columns packed with commercially available materials have good separabilities. A 10 cm \times 0.25 mm I.D. column yields around 7000 theoretical plates, comparable with a conventional HPLC column. A flow injection system which can load a small volume of sample solution (less than 0.05 μ l) will be required for the further development of HPLC on such columns.

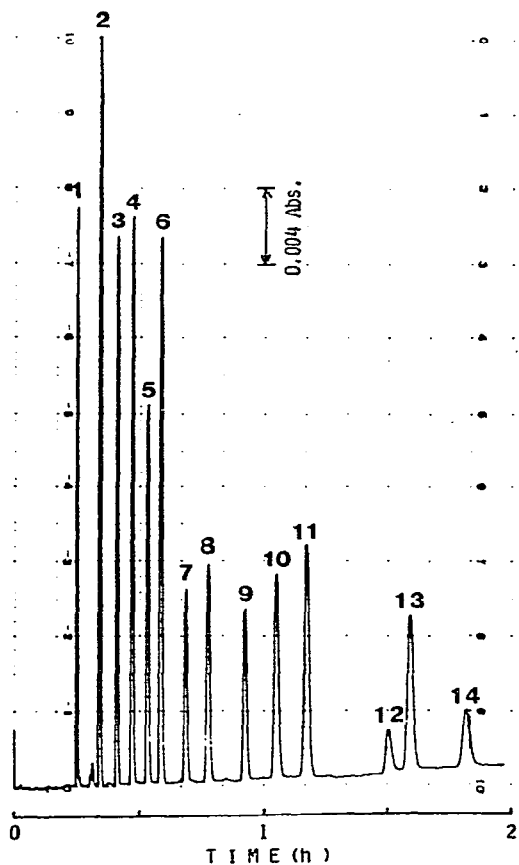


Fig. 7. Separation of polynuclear aromatic hydrocarbons on a 30 cm \times 0.25 mm I.D. column. Mobile phase: acetonitrile-water (70:30); flow-rate 1.4 μ l/min. Samples as in Fig. 4, injection volume 0.09 μ l. Temperature: 30°C. Detection wavelength: 254 nm.

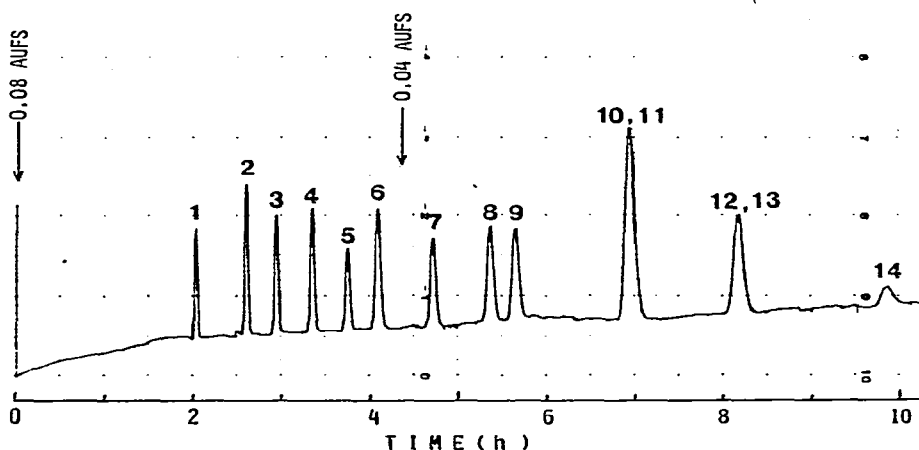


Fig. 8. Separation of polynuclear aromatic hydrocarbons on an 80 cm \times 0.25 mm I.D. column. Mobile phase: acetonitrile-water (80:20); flow-rate 0.35 μ l/min. Samples as in Fig. 4, injection volume 0.06 μ l. Temperature: 26°C. Detection wavelength: 254 nm.

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