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MICRO-HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY WITH LONG MICRO-PACKED FLEXIBLE FUSED-SILICA COLUMNS

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SUMMARY

Coupling of several 20 cm × 0.25–0.35 mm I.D. micro packed flexible fused silica columns in series is a simple technique for attaining high efficiencies in micro high-performance liquid chromatography. Reversed-phase and gel permeation chromatography using 40–140-cm columns are demonstrated. A 140-cm column packed with G 1000H (5 μm) attained *ca.* 100,000 plates in gel permeation chromatography. These columns were employed to resolve complex polychlorinated biphenyl mixtures or the constituents of water.

INTRODUCTION

For the liquid chromatographic analysis of complex mixtures in the environment or *in vivo*, high efficiency in terms of the theoretical plate number plays an important role as well as optimization of the selectivity of liquid chromatography (LC). A greater number of theoretical plates is generally produced by a longer column[1–3]. Scott and Kucera[1,2] prepared 1 m × 1 mm I.D. microbore packed columns and obtained longer columns (10–14 m) by coupling them in series. Nearly 30,000 plates were produced with a 1-m column in the reversed-phase system, 160,000 plates were produced with a 10-m column in the normal-phase system and 650,000 plates were produced with a 14-m column in gel permeation chromatography[2]. The pressure drop across the column and the time required for the analysis were much greater than for conventional high-performance LC (HPLC).

We have recently examined the employment of flexible fused silica tubing as the column material in micro-HPLC[4] when we found that a 10 cm × 0.25 mm I.D. column attained *ca.* 7000 theoretical plates. In that work[4], the effect of column length on column efficiency was investigated and nearly the same efficiency in terms of height equivalent to a theoretical plate (HETP) was attained with 5–30 cm columns, while a slightly poorer efficiency was observed for an 80-cm column owing to non-uniformity in the packing. However, it may be expected that a desired efficiency can be generated with long columns coupled in series.

A long straight column causes a large separation between the detector and the pump of the chromatograph. Thus, it is desirable for a long column to be coiled.

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However, it has been noted that the coil should not be less than 12 cm in diameter since a reduction in coil diameter causes a significant reduction in efficiency[2]. Flexibility of a fused-silica tubing facilitated adjustment of the coil diameter after packing. Even a long fused-silica tubing column is easy to handle.

In this article, high-resolution flexible fused-silica columns coupled in series were prepared and used for the analysis of complex mixtures by reversed-phase and gel permeation chromatography.

EXPERIMENTAL

The liquid chromatograph was assembled from a Micro Feeder (Azumadenki Kogyo, Tokyo, Japan) with a 250-μl gas-tight syringe as a pump, a JASCO micro valve injector (0.02 μl, Japan Spectroscopic, Tokyo, Japan), a micro pre-column, a micro packed fused silica column, a column oven, a UVIDEC-100 (JASCO) UV spectrometer with a modified flow cell and a back-pressure pump. Fused-silica columns were prepared by the method reported previously[4]. TSK-GEL G 1000H (5 μm), Toyo Soda, Tokyo, Japan) and silica ODS SC-01 (5 μm, JASCO) were selected as packings for gel permeation and reversed-phase chromatography, respectively. Columns were connected by stainless-steel tubing, 0.13 mm I.D., 0.31 mm O.D. and 4–5 mm length, as shown in Fig. 1. The connection volume was only 0.05–0.06 μl and the connecting system shown in Fig. 1 endured 80–100 kg/cm^2. The micro precolumn was composed of PTFE tubing, *ca.* 10 mm × 0.2 mm I.D., packed with Amberlite XAD II and was employed for collecting the organic constituents of water using the technique reported previously[5]. The column oven was constructed in our laboratory and comprised asbestos boards equipped with a heater and a fan. The temperature was adjusted by altering the applied voltage with a sliding rheostat. A micro flow cell was composed of quartz tubing, 1.5 mm × 0.17 mm I.D.. Back-pressure was applied to prevent vaporization of the mobile phase even when the chromatograph was operated at elevated temperatures as described previously[6]. Samples were injected using a modified micro valve injector[7]. All reagents were purchased from Wako (Osaka, Japan) or Tokyo Chemical Industry (Tokyo, Japan). Fused-silica tubings were obtained from Gasukuro Kogyo (Tokyo, Japan) or Scientific Glass (North Melbourne, Australia).

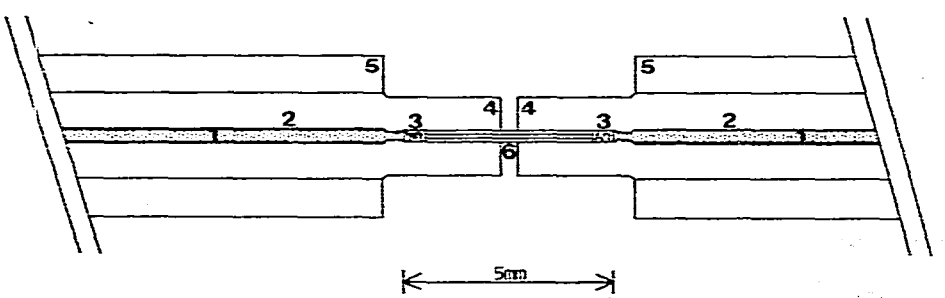

Fig. 1. Schematic diagram of the ends of columns and the connecting capillary tubing. 1 = Packing material; 2 = fused-silica tubing; 3 = quartz wool; 4 = PTFE tubing, 0.2 mm I.D. and 2 mm O.D.; 5 = PTFE tubing, 2 mm I.D. and 4 mm O.D.; 6 = stainless-steel capillary tubing, 0.13 mm I.D. and 0.31 mm O.D.

RESULTS AND DISCUSSION

Reversed-phase chromatography

In previous work[4], 5–30 cm × 0.25 mm I.D. fused-silica columns packed with silica ODS SC-01 (5 μm) gave nearly the same HETP value, 14–20 μm. Thus, 20 cm × 0.25 mm I.D. was adopted as the column dimension in this work, because of the simplicity of preparation.

Table I shows the efficiency of 20-cm columns and coupled columns using aromatic hydrocarbons as test samples and acetonitrile–water (7:3) as the mobile phase. Dependence of HETP on the flow-rates of the mobile phase was low in the region between 0.83 and 3.3 μl/min. Columns of length 20 cm generally produced 10,400–14,100 theoretical plates. The relative standard deviation of HETP with ten 20-cm columns was *ca.* 9%. A 9.9-cm column and coupled columns (39.6 and 59.1 cm) also gave nearly the same HETP value as the 20-cm columns, which indicates that extra-column band broadening in the connection, injection and detection parts is negligibly small.

TABLE I

COLUMN PERFORMANCE OF FLEXIBLE MICRO COLUMNS

Column: 0.25 mm I.D., packed with silica ODS SC-01 (5 μm). Mobile phase: acetonitrile–water (7:3). V_R = Retention volume; N = number of theoretical plates; HETP = height equivalent to a theoretical plate.

Column length (cm)	*Flow-rate (μl/min)*	*Column performance*					
		Biphenyl			*Pyrene*		
		V_R (μl)	N	*HETP (μm)*	V_R (μl)	N	*HETP (μm)*
9.9	1.11	11.7	7100	14	24.8	7000	14
20.0	1.04	23.3	10,400	19	42.0	12,300	15
19.7	1.04	23.5	12,400	16	47.2	12,200	16
19.9	1.04	24.0	12,400	16	47.9	10,500	19
19.5	1.04	23.1	12,300	16	47.0	12,500	16
19.5	1.04	23.0	12,200	16	46.3	12,100	16
19.7	1.04	23.0	14,000	14	45.7	14,100	14
19.9	1.04	24.3	14,000	14	49.4	14,100	14
19.9	2.08	24.0	11,700	17	45.8	13,100	15
20.0	2.08	23.0	10,800	19	44.5	12,500	16
19.9	2.08	23.1	11,700	17	43.0	13,100	15
39.6 (19.7 + 19.9)	1.04	47.3	28,400	14	96.2	23,600	17
59.1 (19.7 + 19.9 + 19.5)	1.04	69.6	40,500	15	142.6	40,600	15

Fig. 2 shows the linear relationship between the theoretical plate number and column length. A desired column efficiency can be attained with the columns coupled in series. On the other hand, a single 80-cm column gave a slightly poorer result (HETP = 30–37 μm).

The pressure drop across the 59.1-cm column under the operating conditions

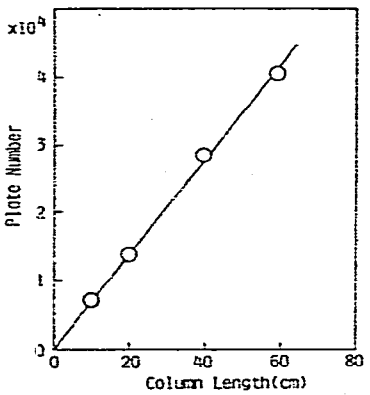

Fig. 2. Relationship between the theoretical plate number and column length. Column, 0.25 mm I.D., packed with SC-01 (5 μm); mobile phase, acetonitrile–water (7:3); flow-rate, 1.04 μl/min; sample, biphenyl; temperature, ambient.

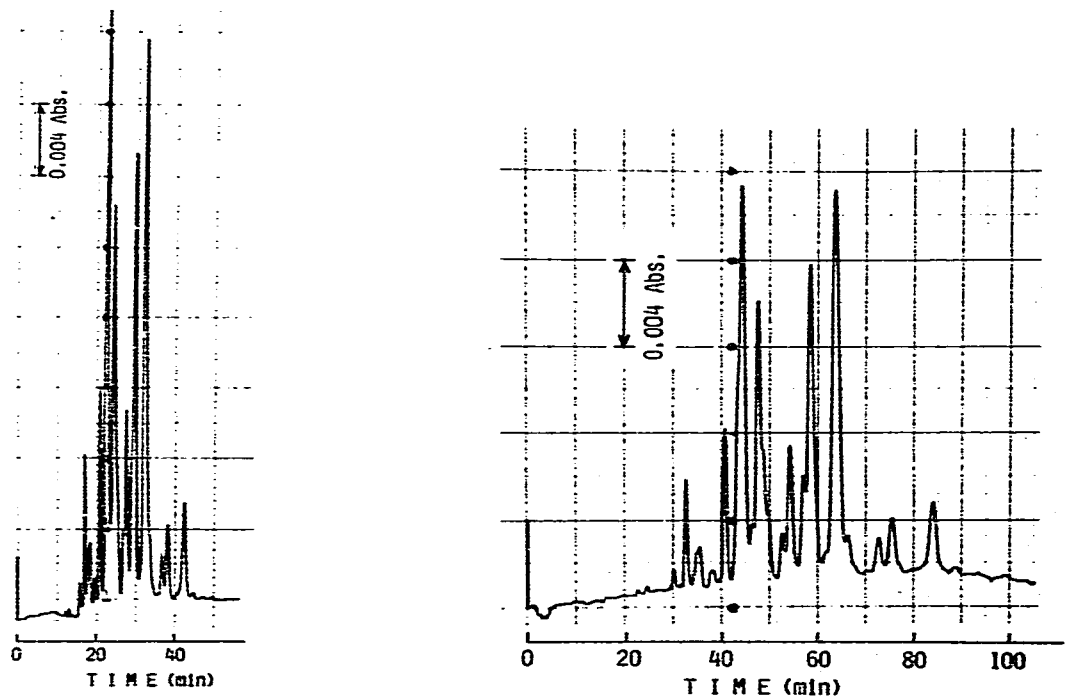

Fig. 3. Separation of PCB mixtures on a 20.0-cm column. Column, 20.0 cm × 0.25 mm I.D., packed with SC-01 (5 μm); mobile phase, acetonitrile–water (8:2); flow-rate: 1.39 μl/min; sample, 1.8% of PCB-48; (Tokyo Chemical Industry, chlorine content *ca.* 48%) sample size, 0.02 μl; temperature, 27°C; wavelength of UV detection, 254 nm.

Fig. 4. Separation of PCB mixtures on a 39.9-cm column. Column, 39.9 cm × 0.25 mm I.D., packed with SC-01 (5 μm). Other operating conditions as in Fig. 3.

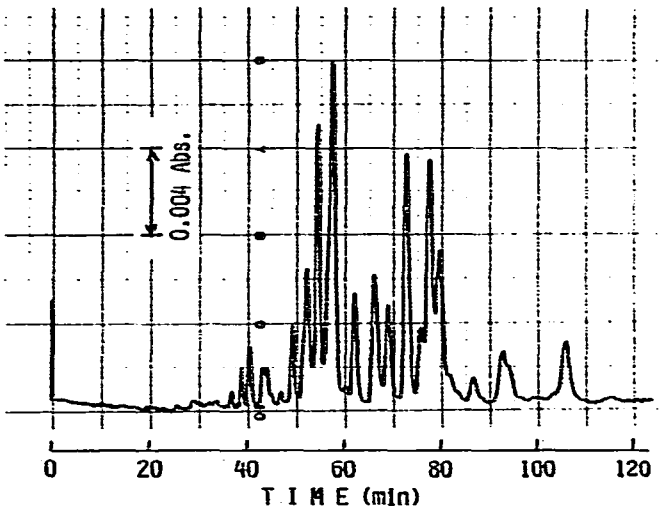

Fig. 5. Separation of PCB mixtures on a 39.5-cm column at elevated temperature. Column: 39.5 cm × 0.25 mm I.D., packed with SC-01 (5 μm); mobile phase, acetonitrile–water (7:3); temperature, 62°C. Other operating conditions as in Fig. 3.

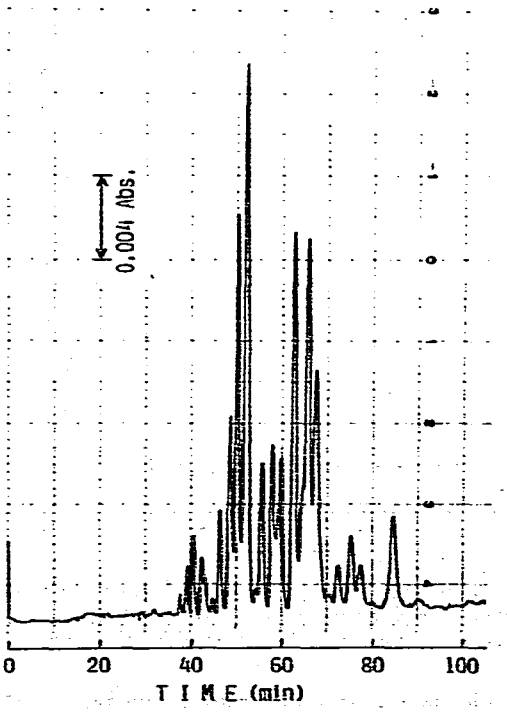

Fig. 6. Separation of PCB mixtures on a 59.1-cm column at elevated temperature. Column, 59.1 cm × 0.25 mm I.D., packed with SC-01 (5 μm); mobile phase, acetonitrile–water (7:3); sample, 2.5% of PCB-48; temperature, 90°C. Other operating conditions as in Fig. 3.

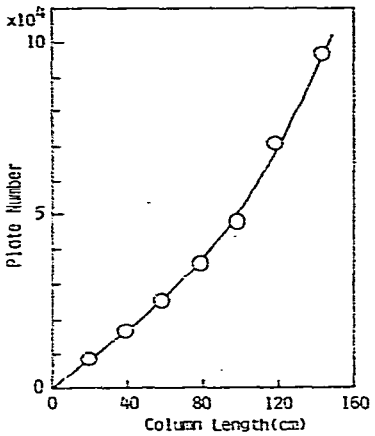

Fig. 7. Relationship between the theoretical plate number and column length. Column, 0.35 mm I.D., packed with G 1000H (5 μm); mobile phase, tetrahydrofuran; flow-rate, 1.04 μl/min; sample, dimethyl phthalate; temperature, ambient.

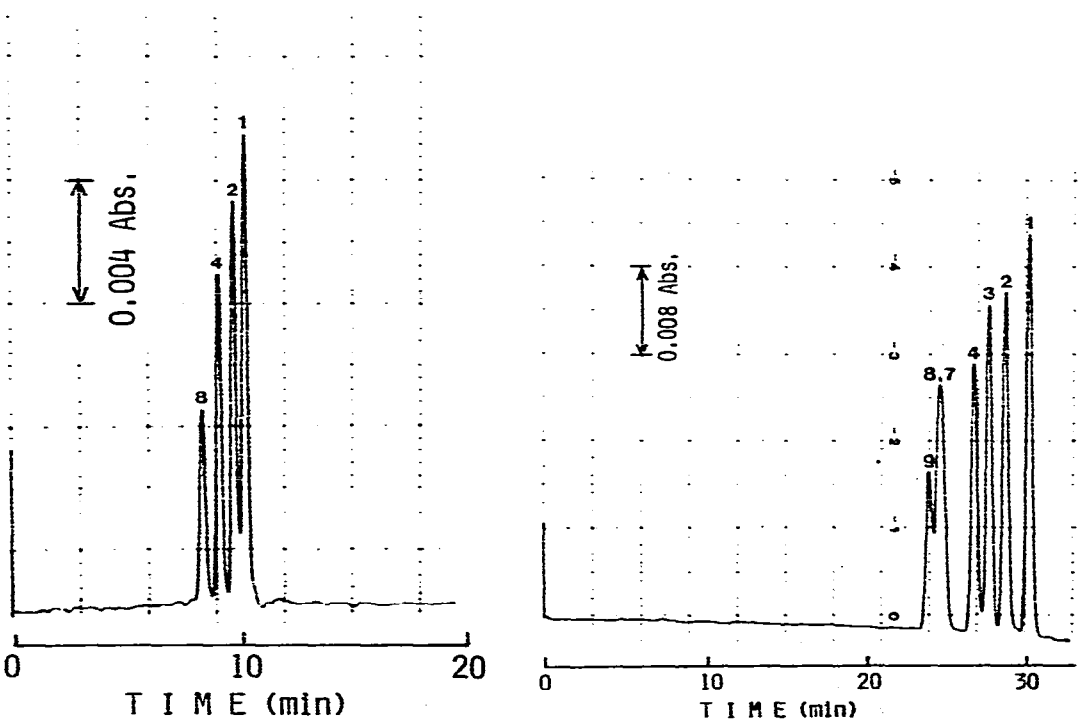

Fig. 8. Separation of phthalates on a 19.4-cm column. Column, 19.4 cm × 0.35 mm I.D., packed with G 1000H (5 μm); flow-rate, 1.04 μl/min. Samples: 8 = di-2-ethylhexyl phthalate; 4 = di-*n*-butylphthalate; 2 = diethyl phthalate; 1 = dimethyl phthalate, each 0.05%. Sample size, 0.02 μl; temperature, ambient; wavelength of UV detection, 235 nm.

Fig. 9. Separation of phthalates on a 57.8-cm column. Column, 57.8 cm × 0.35 mm I.D., packed with G 1000H (5 μm). Samples: 9 = dinonyl phthalate; 8 = di-2-ethylhexyl phthalate; 7 = diheptyl phthalate; 4 = di-*n*-butyl phthalate; 3 = di-*n*-propylphthalate; 2 = diethylphthalate; 1 = dimethylphthalate, each 0.2%. Other operating conditions as in Fig. 8.

indicated in Table I was *ca.* 60 kg/cm^2. The pumping, injecting and connecting systems employed in this work endured 60–80 kg/cm^2. Thus, the chromatograph should be run at lower flow-rates if longer columns are employed, leading to an increase in the analysis time.

Figs. 3–6 demonstrate the analyses of polychlorinated biphenyl (PCB) mixtures on columns of different lengths. Figs. 3 and 4 show separations at room temperature (27°C) on 20.0- and 39.9-cm columns and Figs. 5 and 6 show separations at elevated temperature on 39.5- and 59.1-cm columns, respectively. The longer the column, the more constituents are resolved. The chromatograms obtained on 40-cm columns shown in Figs. 4 and 5 suggest that selectivity at elevated temperatures is higher than at room temperature, which encouraged us to employ the water-rich mobile phase at elevated temperature. In addition, operating at elevated temperature generally decreases the viscosity of the mobile phase, leading to a decrease in the inlet pressure. Thus, operation at elevated temperature facilitates the employment of longer columns and/or separation at higher flow-rates.

Gel permeation chromatography

Several studies on micro-scale gel permeation chromatography have been reported[1,2,8,9], in which 0.5–1-mm bore columns are employed. In this work, TSK-GEL G 1000H (5 μm) and a 0.35-mm I.D. fused-silica tubing were selected as packing and column material, respectively, for gel permeation chromatography.

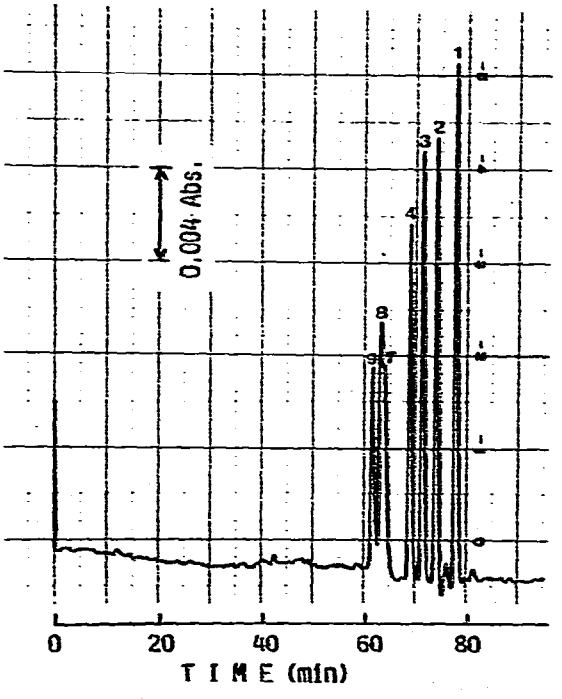

Fig. 10. Separation of phthalates on a 142.6-cm column. Column, 142.6 cm × 0.35 mm I.D., packed with G 1000H (5 μm); samples as in Fig. 9. Other operating conditions as in Fig. 8.

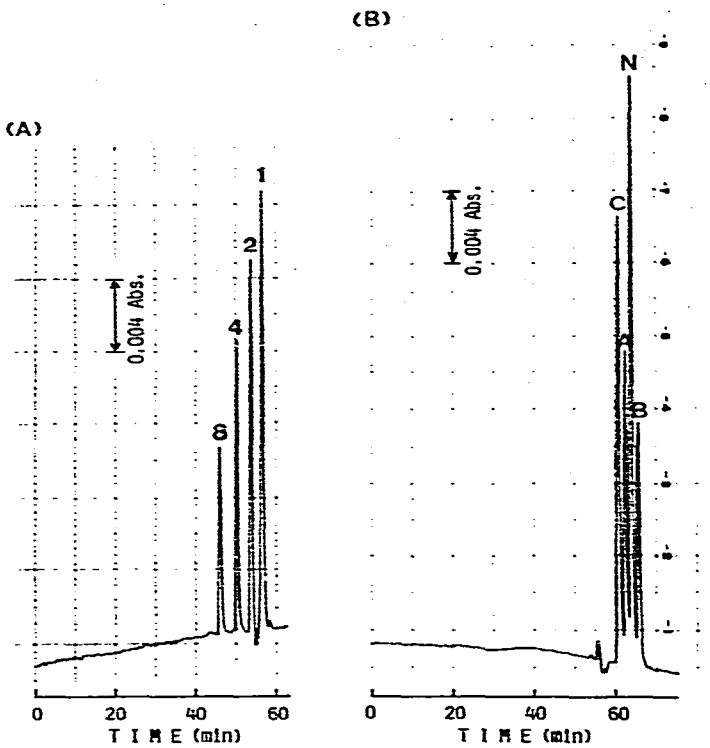

Fig. 11. Separations of phthalates and aromatic hydrocarbons on a 102.8-cm column. Column, 102.8 cm × 0.35 mm I.D., packed with G 1000H (5 μm); mobile phase, tetrahydrofuran; flow-rate, 1.04 μl/min. Samples: (A) phthalates, as in Fig. 8 except for the concentration, each 0.2%; (B) aromatic hydrocarbons: C = 0.034% (w/v) of chrysene; A = 0.0087% of anthracene; N = 0.38% of naphthalene; B = 2.6% of benzene. Sample size, 0.02 μl; temperature, ambient; wavelength of UV detection, (A) 235 nm, (B) 254 nm.

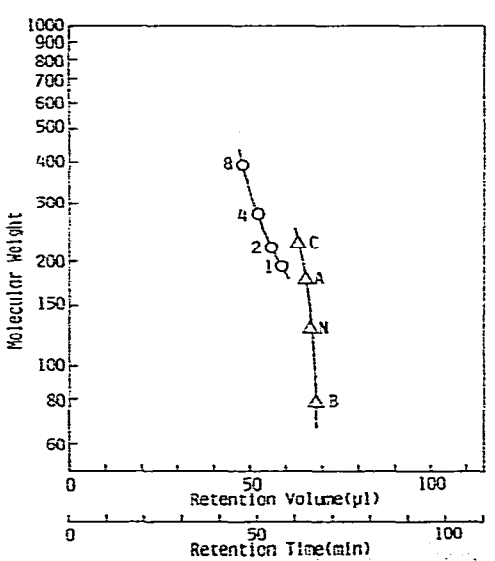

Fig. 12. Relationship between the logarithm of molecular weight and retention volume (or time). Operating conditions as in Fig. 11.

The efficiency of columns coupled in series was examined using phthalates and aromatic hydrocarbons as the test samples and tetrahydrofuran as the mobile phase. Fig. 7 shows the relationship between the theoretical plate number and the column length. The theoretical plate number increases with increasing column length and the relation deviated upwards from linearity. Nearly 100,000 theoretical plates were produced for dimethyl phthalate on a 140-cm column at a retention time of 80 min.

Figs. 8–10 show typical separations of phthalates on columns of different lengths. As the column length increases, the resolution of the solutes becomes greater, *e.g.*, dimethyl and diethyl phthalates are not completely resolved on a 19.4-cm column, whereas baseline separations of these two phthalates are attained on 57.8- and 142.6-cm columns. Fig. 11 shows separations of phthalates and aromatic hydrocarbons on a 102.8-cm column and Fig. 12 shows the relationship between retention volume (or time) and the logarithm of the molecular weight (MW). The compound of higher MW elutes before that of lower MW in the homologues, owing to simple size

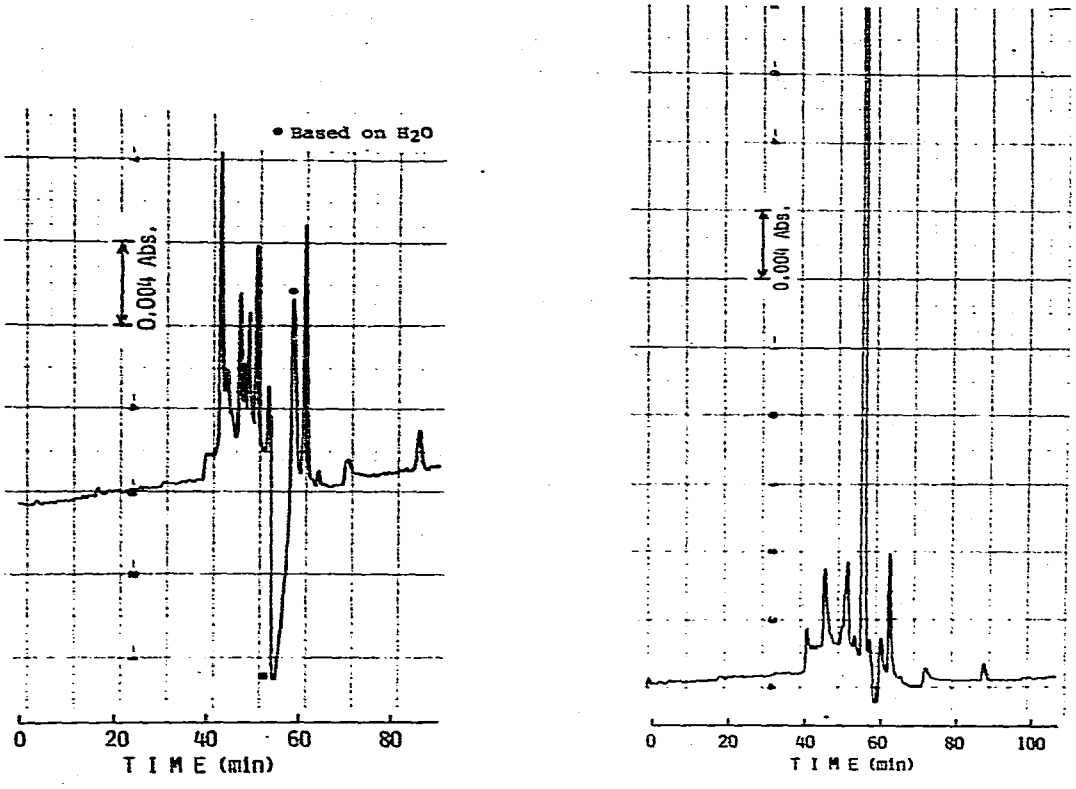

Fig. 13. Analysis of constituents of water. Column, 102.8 cm × 0.35 mm I.D., packed with G 1000H (5 μm); mobile phase, tetrahydrofuran; flow-rate, 1.04 μl/min; pre-column, 1 cm × 0.2 mm I.D., packed with Amberlite XAD II (10 μm); sample, 1 ml of deionized water; temperature, ambient; wavelength of UV detection, 235 nm.

Fig. 14. Analysis of extracts in water from coal. Sample, 1 ml of water in contact with powdered coal. Operating conditions as in Fig. 13.

exclusion. Retention of solutes also depends on the structure of the solutes, *e.g.*, chrysene (MW = 228) elutes after diethyl phthalate (MW = 222) and dimethyl phthalate (MW = 194). Compact compounds generally elute after less compact compounds of the same MW.

High-resolution flexible fused silica columns were applied to some analyses of water samples. Fig. 13 demonstrates the analysis of the organic constituents of de-ionized water. The sample was passed through a pre-column packed with Amberlite XAD II (10 μm) prior to the chromatographic run. After the pre-column containing the organic constituents had been dried *in vacuo*, it was connected to the separation column. A number of peaks were resolved due to the difference in size, although residual water in the pre-column interfered with the detection of some solutes.

The analysis of extracts in water from coal is shown in Fig. 14. MWs of peaks can be estimated by the calibration data indicated in Fig. 12.

CONCLUSION

Micro-packed flexible fused-silica columns coupled in series produced desired efficiencies. A range of 20,000 to 100,000 theoretical plates could be attained on these columns for reversed-phase and gel permeation chromatography, and allowed the resolution of complex mixtures. The technique will be useful for the analysis of various real samples.

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