Journal of Chromatography, 477 (1989) 161–168
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 21 541

MULTIDIMENSIONAL PACKED CAPILLARY COLUMN SUPERCRITICAL-FLUID CHROMATOGRAPHY USING A FLOW-SWITCHING INTERFACE

K. M. PAYNE and I. L. DAVIES[a]
Department of Chemistry, 226 Eyring Science Centre, Brigham Young University, Provo, UT 84602 (U.S.A.)
K. D. BARTLE
School of Chemistry, The University of Leeds, Leeds, LS2 9JT (U.K.)
and
K. E. MARKIDES and M. L. LEE*
Department of Chemistry, 226 Eyring Science Centre, Brigham Young University, Provo, UT 84602 (U.S.A.)

SUMMARY

A two-dimensional supercritical-fluid chromatographic (SFC–SFC) system with a flow-switching interface was used with 250 μm I.D. packed capillary columns to separate polycyclic aromatic hydrocarbons (PAHs) in a standard coal tar extract. A solvent-venting injection technique was also used which allowed the injection of several microliters of sample. Compared to open-tubular columns, packed capillary columns provided shorter analysis times and higher sample capacities; compared to conventional size packed columns, they provided higher efficiencies and lower volumetric flow-rates, allowing the total effluent to be introduced into a flame-based detector without the need for splitting.

The performance of packed capillary SFC–SFC was compared to that of open-tubular capillary SFC–SFC. In the first dimension, separation according to the number of aromatic rings was achieved as desired within 30 min, two times faster than previously reported for an open tubular column using the same sample. However, the selectivity and efficiency obtained in the second dimension, using a packed capillary, was not sufficient to fully separate all of the PAH isomers.

INTRODUCTION

In supercritical-fluid chromatography (SFC) as well as gas chromatography (GC), open tubular capillaries are associated with high efficiency. Because of their openness, columns which are longer than several meters can be used to provide a

[a] On leave from the School of Chemistry, The University of Leeds, Leeds, LS2 9JT, U.K.

0021-9673/89/$03.50

large number of theoretical plates. Capillary SFC, however, does not match the speed of analysis offered by packed-column SFC. Mass transfer effects, as a function of linear velocity, are more favorable for packed columns. The Van Deemter curve above the optimum linear velocity for a packed column has less slope than that for an open-tubular column, resulting in a wider range of useable linear velocities with minimal loss of efficiency. It is this fact that allows packed columns to provide faster analysis times while still preserving resolution.

Commonly used packed columns in SFC vary between 250 μm and 4.6 mm I.D. Columns of 1 mm I.D. (microbore) and larger require much greater volumetric flow-rates than packed capillary columns. When operated near optimum conditions, up to several l min^{-1} of gas can flow from the end of a microbore column. An advantage of SFC is the compatibility of the mobile phase used (generally CO_2) with flame-based, highly sensitive detectors, such as the flame ionization detector. In order not to extinguish the flame when using these larger packed columns with mobile phase flow-rates near their optimum, some of the effluent must be split. Splitting of the effluent, to the detector, not only can create discrimination problems, but the increased sample capacity obtained by using packed columns is diminished before the sample reaches the detector.

Packed capillaries with 100–250 μm I.D. provide a convenient compromise between open-tubular capillaries and conventional packed columns. The low volumetric flow-rates of packed capillary columns are comparable to open-tubular capillary flow-rates, yet at the same time, packed capillaries possess much greater sample capacities than open-tubular capillary columns. In comparison to conventional packed columns, packed capillaries provide higher permeability, allowing longer columns to be packed for greater total efficiency. The lower volumetric flow-rates of packed capillaries permit the total effluent to be introduced to mass-flow-sensitive detectors. The small elution volume provides more sensitive detection in concentration-dependent detectors and allows sampling of narrow bands during heart cutting in multidimensional systems[1].

The selectivity and resolving power of a single column is often inadequate for the chromatographic analysis of complex samples. By coupling two chromatographic columns of different selectivities in tandem, and with the use of heart-cutting to selectively transfer fractions of the column effluent from the primary column into the secondary column, dramatic improvements in peak capacity and resolution can be realized[2–4].

In this study, a multidimensional packed capillary SFC–SFC system based on a simple flow-switching interface was evaluated. This system was applied to the separation of polycyclic aromatic hydrocarbon (PAH) components in a standard coal tar extract, a sample which was recently separated using open-tubular SFC–SFC[5].

EXPERIMENTAL

The SFC–SFC system, used in this study, was similar in design to an open-tubular SFC–SFC system previously described[5], with minor modifications for packed capillary use. A schematic diagram of the modified system is shown in Fig. 1.

A ten-port valve (Valco, Houston, TX, U.S.A.) was used to direct the CO_2 mobile phase through both columns in series or only to the second column (Col_2).

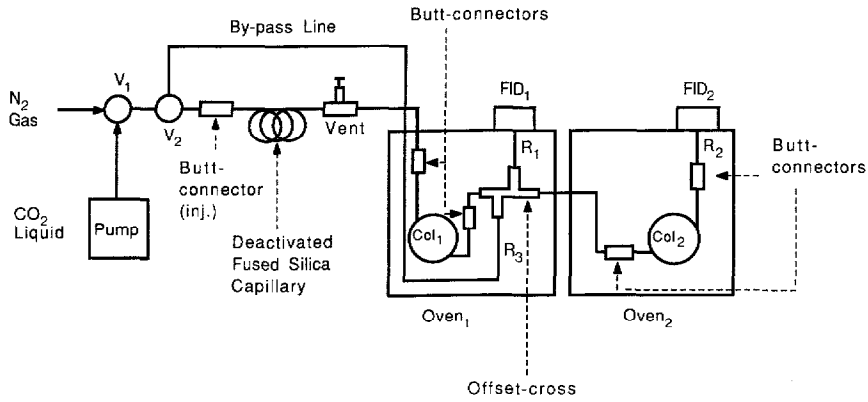

Fig. 1. Schematic diagram of the multidimensional microcolumn SFC–SFC system using a flow-switching interface for packed capillary columns.

The bypass line connecting the bypass valve (V_2) with the offset-cross flow-switching interface was a 20 cm × 25 μm I.D. untreated fused-silica capillary (Polymicro Technologies, Phoenix, AZ, U.S.A.). The end of the bypass line was butt-connected within the first oven to a frit restrictor in order to control the flow-rate to Col_2 and to avoid rapid detector signal changes during heart-cutting. The end of the restrictor was inserted into the offset-cross interface[5].

A solvent-venting technique was employed which allowed the introduction of variable amounts of sample onto the column[6]. The injections were made by inserting an on-column syringe directly into a deactivated fused-silica capillary (1.5 m × 100 μm I.D.) that was purged with nitrogen gas to remove solvent, and then with mobile phase to initiate the chromatographic separation.

Vespel ferrules (Alltech, Deerfield, IL, U.S.A.) were used to secure the 250 μm

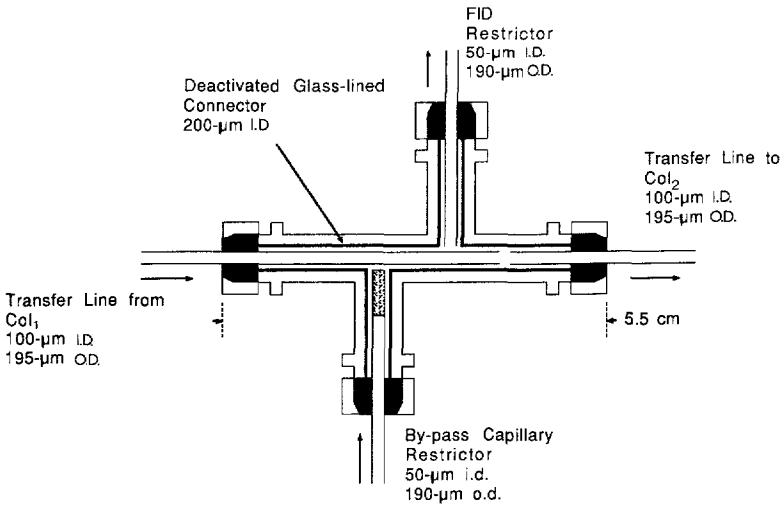

Fig. 2. Details of the offset-cross interface between the primary and secondary columns of the SFC–SFC system of Fig. 1.

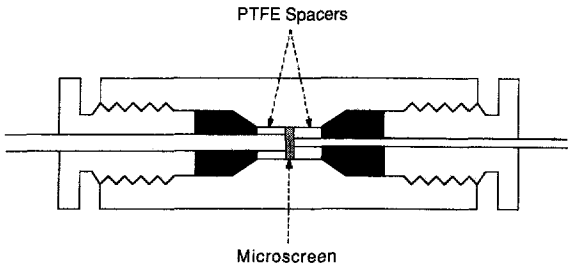

Fig. 3. Schematic diagram of the zero-dead-volume union used for packed capillary connections.

I.D. analytical column within Valco zero-dead-volume unions to either 100 μm I.D. cyanopropyl-deactivated[7] fused-silica transfer lines [appropriate lengths of 100 μm I.D. deactivated transfer lines carried effluent from the vent T-piece to the head of the primary column (Col_1), from Col_1 to the offset-cross (Fig. 2), and from the offset-cross to Col_2 through the copper tube which connected the two ovens] or to 50 μm I.D. frit restrictors. Small segments of 200 μm I.D. PTFE tubing (Chrompack, Middelburg, The Netherlands) were used to eliminate potential dead volumes in the zero-dead-volume unions (Fig. 3). Stainless steel microscreens (Mectron, City of Industry, CA, U.S.A.) were used to support the capillary column packed beds.

Two 250 μm I.D. slurry-packed fused-silica capillary columns were used in this study. Col_1 was 25 cm in length and packed with 7 μm diameter, 300 Å pore, aminosilane-bonded silica (NH_2-silica, Nucleosil, Machery-Nagel, Düren, F.R.G.). Col_2 was 55 cm in length and packed with 5 μm diameter, 300 Å pore, silica packing material which had been modified with a polyoctylhydrosiloxane via a dehydrocondensation reaction procedure developed in our laboratory[8].

$Oven_1$ and $Oven_2$ were held isothermal at 100 and 120°C, respectively. The density programs for both columns began at 0.35 g ml^{-1} and, after 2 min, were ramped at 0.008 g ml^{-1} min^{-1} to a density of 0.75 g ml^{-1}. The linear velocities were 0.9 cm s^{-1} for Col_1 and 0.6 cm s^{-1} for Col_2. A Van Deemter curve was generated to determine the optimum linear velocity for the packed capillary columns (Fig. 4). Column dead times were determined by injecting *n*-hexane and venting for only 15 s

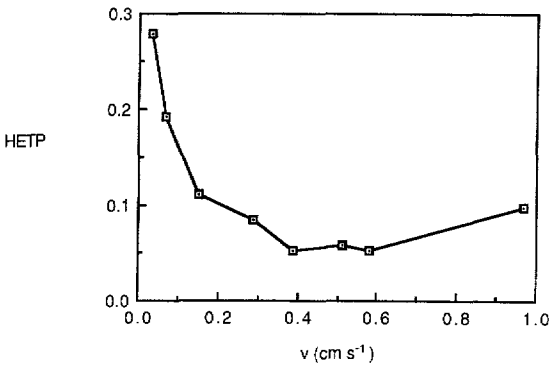

Fig. 4. Plot of a Van Deemter curve generated from a 42 cm × 250 μm I.D. capillary column (packed with 5 μm diameter, 300 Å pore silica particles) under isobaric conditions: 100°C, 150 atm, CO_2 mobile phase.

in order to introduce a small detectable amount of solvent onto the column. The results verified that Col_1 and Col_2 were operated at about 2.0 and 1.5 times their optimum linear velocities, respectively.

The sample analyzed was a National Institute of Standards and Technology standard reference material (SRM) 1597[9], which is a natural, complex, combustion-related mixture of PAHs isolated from a crude coke oven tar[10]. A volume of 1.2 μl was injected without dilution, providing 4 times the mass of solutes that were injected by using the open-tubular system[5]. This quantity of sample was too large to be injected directly onto an open-tubular capillary column.

RESULTS AND DISCUSSION

The flow-switching interface employed in this work was found to be inadequate for the combination of packed capillary/open-tubular capillary column SFC–SFC[5]. This is because the optimum volumetric flow-rates required for each column type differ greatly, and they could not be independently controlled with this type of interface. It was suggested from previous work[5] that two packed capillary columns could be used in series, thereby avoiding these volumetric flow difficulties.

When initially making fractionation cuts, a rapid signal change was noticed as a result of dramatic pressure and flow changes. A restrictor was therefore placed at the end of the by-pass line as it entered the offset-cross to dampen these changes. This modification was unnecessary in previous open-tubular SFC–SFC work[5] due to the steady back pressures maintained by the long 50 μm I.D. capillary columns.

Using two open-tubular columns in SFC–SFC, the total time required to elute the SRM 1597 sample from both columns without fractionation, was about 4 h. To fractionate the sample into individual groups required approximately 11 h. The use of

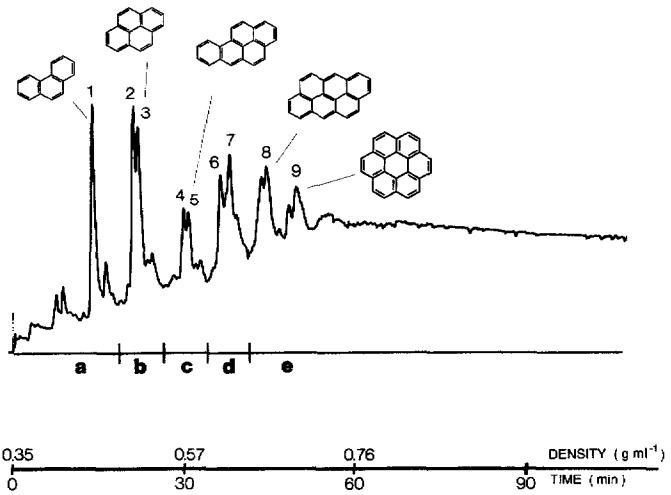

Fig. 5. Packed capillary column chromatogram (first dimension) of a standard coal tar extract (SRM 1597), using the system of Fig. 1. Conditions: see text. Peak identifications: 1 = phenanthrene; 2 = fluoranthene; 3 = pyrene; 4 = benz[*a*]anthracene; 5 = chrysene; 6 = benzofluoranthene isomers; 7 = benzopyrene isomers; 8 = anthanthrene; 9 = coronene.

an open-tubular liquid crystal column in the second dimension to provide shape selectivity, allowed the separation of various isomers including chrysene, triphenylene, benz[*a*]anthracene, and the benzofluoranthenes. Considerable time was needed to elute the sample from the first column, and over 1 h passed before the first peak was detected at the second flame ionization detector. The first column provided more efficiency than was really needed; only a crude PAH ring-number separation was necessary. A short primary column that could provide adequate but fast group type separation would be preferable.

Using packed columns in SFC–SFC, the SRM sample was separated into ring-number fractions in the first dimension in less than 1 h (Fig. 5). The fractionation of

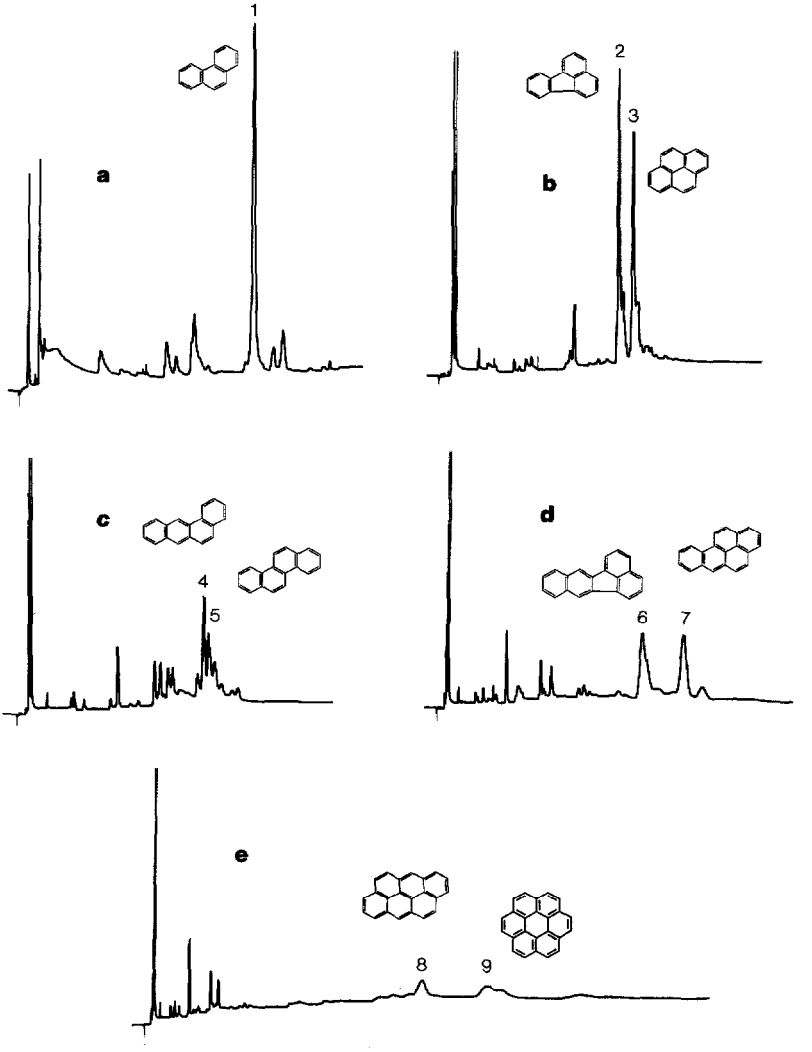

Fig. 6. Packed capillary column SFC–SFC chromatograms from the second dimension (fractions a–e indicated in Fig. 5) of a standard coal tar extract (SRM 1597) by using the system of Fig. 1. Conditions and peak identifications as in Fig. 5.

TABLE I

RELATIVE RETENTION TIMES FOR SELECTED PAHs IN SRM 1597

Detection with flame ionization detector (FID).

Peak No.	*PAH*	*MW*	*Relative retention time*[a]	
			FID_1	FID_2
1	Phenanthrene	178	1.00	1.00
2	Fluoranthene	202	1.52	1.24
3	Pyrene	202	1.56	1.30
4	Benz[*a*]anthracene	228	2.14	1.45
5	Chrysene	228	2.20	1.47
6	Benzofluoranthenes	252	2.61	1.65
7	Benzopyrenes	252	2.73	1.83
8	Anthanthrene	276	3.20	2.26
9	Coronene	300	3.57	2.40

[a] Retention relative to phenanthrene.

the sample into five groups, followed by further resolution in the second dimension, was completed within 5 h (Fig. 6). In all, the analysis was completed in less than half the time required for the coupled open-tubular column system. Relative retention data are given in Table I. Good group separation, according to ring number, was quickly achieved by using the NH_2-silica phase in Col_1. Isomers such as chrysene and benz[*a*]anthracene were resolved by using Col_2. However, the benzofluoranthene and benzopyrene isomers were far from being satisfactorily resolved. The fractionation system itself worked well, but greater resolution in the second dimension would be required to achieve the desired separation of isomers. The elution of coronene from Col_2 did not occur until 30 min after the end of the density program (0.76 g ml^{-1}). This is in contrast to the results from the coupled open-tubular system where over 1 h passed before coronene eluted from the second dimension. The limiting factor controlling efficiency in both cases was the compromise chosen between high temperature in order to achieve better efficiency and the pressure limit of the syringe pump (400 atm).

CONCLUSIONS

In the first dimension of multidimensional chromatography, a fast, group-type separation is often the primary goal. In addition, large sample capacity is important for analyses in which the measurement of minor constituents of a sample is desired. These are two broad application areas in which a packed capillary column would be better suited for use in the first dimension than an open-tubular capillary column.

The second dimension must provide sufficient selectivity and efficiency to fully resolve the components of interest. For this particular sample, the liquid crystal stationary phase probably would be the best choice, whether an open-tubular capillary column or a longer packed capillary column were used. As was previously mentioned, the option of using a packed capillary column in tandem with an open-tubular

capillary column was not possible in this study due to the limitations imposed by the offset-cross interface; a rotary valve interface would allow independent control of each dimension, thereby facilitating such a combination[11].

ACKNOWLEDGEMENTS

This work was supported by the Gas Research Institute (Contract No. 5084-260-1129) and by the State of Utah Centers of Excellence Program (Contract No. 872279).

REFERENCES

1 F. J. Yang, in F. J. Yang (Editor), *Microbore Column Chromatography: A Unified Approach to Chromatography*, Marcel Dekker, New York, 1989, p. 1.
2 J. C. Giddings, *Anal. Chem.*, 56 (1984) 1258A.
3 J. C. Giddings, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 10 (1987) 319.
4 G. Schomburg, H. Husmann, E. Hubinger and W. A. Konig, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 7 (1984) 404.
5 I. L. Davies, B. Xu, K. E. Markides, K. D. Bartle and M. L. Lee, *J. Microcolumn Sep.*, 1 (1989) 71.
6 M. L. Lee, B. Xu, E. C. Huang, N. M. Djordjevic, H.-C. K. Chang and K. E. Markides, *J. Microcolumn Sep.*, 1 (1989) 7.
7 K. E. Markides, B. J. Tarbet, C. M. Schregenberger, J. S. Bradshaw, K. D. Bartle and M. L. Lee, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 8 (1985) 741.
8 K. M. Payne, B. J. Tarbet, J. S. Bradshaw, K. E. Markides and M. L. Lee, in preparation.
9 *Certificate of Analysis for Standard Reference Material 1597, Complex Mixture of Polycyclic Aromatic Hydrocarbons from Coal Tar*, National Institute of Standards and Technology, Gaithersburg, MD, 1987.
10 S. A. Wise, B. A. Brenner, G. D. Byrd, S. N. Chesler, R. E. Rebbert and M. M. Schantz, *Anal. Chem.*, 60 (1988) 887.
11 I. L. Davies, Z. Juvancz, K. M. Payne, K. E. Markides, M. L. Lee and K. D. Bartle, *Symposium/Workshop on Supercritical Fluid Chromatography, Snowbird, UT, June 13–15, 1989.*