Journal of Chromatography, 545 (1991) 149–160
Elsevier Science Publishers B.V., Amsterdam

CHROM. 23 229

Factors governing the analytical supercritical fluid extraction and supercritical fluid chromatographic retention of polycyclic aromatic hydrocarbons

JOSEPH REIN, CINDY M. CORK and KENNETH G. FURTON*
Department of Chemistry, Florida International University, University Park, Miami, FL 33199 (USA)

(First received November 13th, 1990; revised manuscript received February 19th, 1991)

ABSTRACT

The molecular connectivity of polycyclic aromatic hydrocarbons (PAHs) correlates well with retention data for several supercritical fluid chromatographic systems. The quantitative effect of microextractor cell geometry on supercritical fluid extraction (SFE) efficiencies of PAHs from octadecyl-bonded sorbents is compared with that seen upon varying the fluid density for the same system. Relative recoveries were increased by up to a factor of two, for coronene, by employing short broad extraction vessels, 1:1 length: I.D., compared to using long narrow vessels, 20:1 length:I.D. The average relative recovery increase was 121% with a large dependence on compound type, increasing *ca.* linearly with fused ring number for the PAHs studied. A similar relative recoveries increase was achievable by increasing the fluid density by 0.12 g ml^{-1} with a small dependence on the compound type (decreasing linearly with fused ring number).

INTRODUCTION

Although Hannay and Hogarth [1] reported the high solvating power of supercritical fluids over a century ago, practical applications of supercritical fluids did not begin to emerge until relatively recently. Two of these techniques are supercritical fluid chromatography (SFC), introduced by Klesper *et al.* [2], and supercritical fluid extraction (SFE), first demonstrated by Zosel [3]. These techniques have received a great deal of attention in recent years as the full potential of supercritical fluids in analytical chemistry has begun to emerge [4,5]. SFE has developed into an important industrial-scale extraction technique as an alternative to distillation and traditional solvent extractions. Recently, the use of SFE on an analytical-scale has received a great deal of attention. SFE can potentially provide more rapid, efficient and selective extractions compared to conventional extraction techniques. Analytical-scale SFE using supercritical CO_2 has been applied to the recovery of a variety of non-polar volatiles and semi-volatiles from solids and adsorbents with up to an order of magnitude increase in the rate of extraction reported compared to conventional methods [6–12].

Supercritical fluids possess unique physicochemical properties which make them attractive as solvents for extraction and chromatography. Supercritical fluids

0021-9673/91/$03.50

have densities (and solvating powers) approaching that of liquids, which are readily changed by varying the temperature and pressure. Supercritical fluids have gaslike transport properties of viscosity and intermediate diffusivity. Additionally, the zero surface tension of supercritical fluids allows for efficient penetration into macroporous materials. SFE can be used on-line, combined with gas, liquid or supercritical chromatography, to provide a complete sample extraction and analysis system, or can be employed off-line, replacing many conventional liquid solvent techniques. On-line methods provide the greatest sensitivity and, in principle, should be more accurate, due to the reduced sample handling. Off-line methods allow the greatest experimental flexibility (*e.g.* fluid type, modifiers, flow-rate, etc.) and the possibility of multiple analysis of the extract regardless of the sample conditions (*e.g.* matrix and concentration). A more thorough discussion of SFE and applications can be found in refs. [13–15].

Extraction of compounds from solid sorbents is controlled by many of the same factors which control SFC retention. These include the affinity of the compounds for the sorbent, the vapor pressure of the compounds, and the solubility and diffusion coefficient of the compounds in the supercritical fluid. Therefore, SFC retention data can be useful in predicting potential SFE recoveries for compounds or classes of compounds, and may provide insight into the processes involved in supercritical extraction. Qualitative correlations between SFC retention and supercritical fluid extractability have been reported [16,17], and, recently, we have demonstrated quantitative correlations for polycyclic aromatic hydrocarbons (PAH's) using octadecyl sorbents [18]. In addition to the above factors, SFE efficiencies are controlled by a complex relationship between many experimental variables, including the density (controlled by pressure), tenperature, extraction cell geometry, compound type, fluid type, fluid modifiers, flow-rate, sample matrix, etc. Although it is well established that, to a first approximation, the solvent power of a supercritical fluid is related to its density, little is known about the relative effects of many of the other controllable variables for analytical-scale SFE. A better understanding of the relative effects of controllable SFE variables is needed to more readily allow SFE extractions to be optimized for maximum selectivity as well as maximum overall recoveries.

EXPERIMENTAL

Apparatus

The extraction apparatus used for this work was constructed from an Isco 260D syringe pump, Valco C6W valve, Fiatron heater and controller, and linear restrictors fabricated from 41 μm I.D. × 362 μm O.D. fused silica (polymicro Technologies, Phoenix, AZ, USA). HPLC guard columns with 2-μm stainless steel frits (Upchurch Scientific, Oak Harbor, WA, USA) were used for the extraction cells. SFC grade carbon dioxide (Scott Specialty Gases, Plumsteadville, PA, USA) was used for all of the extractions. The packed-column supercritical fluid chromatograph used to generate the chromatographic retention data was constructed from a computer (Zenith)-controlled Isco SFC-500 high-pressure syringe pump (Lincoln, NE, USA), Valco C14W 0.5-μl injection valve, Isco 1 mm I.D. microbore C_{18} column, Fiatron column heater and controller (Oconomowoc, WI, USA), and an Isco V^4 UV–Vis detector with 6000 p.s.i. flow cell. Data were collected with an E-Lab Chromatographic control and data acquisition system (OMS Tech, Miami, FL, USA).

Reagents and procedure

Methoxychlor, naphthalene, anthracene, fluorene, pyrene, perylene, benzo[*ghi*]perylene, and coronene were obtained from Aldrich (Milwaukee, WI, USA). Chloroform (Fisher 'Optima') was used to prepare all solutions. The octadecyl coated silica support from 50 1000-mg Prep Sep (Fisher) C_{18} solid-phase extractions cartridges was used to prepare a stock packing used for all of the extractions. An amount of 50 g of the octadecyl packing containing 200 ppm of each PAH was prepared from a chloroform solution with evaporation from a packing flask using a rotary evaporator. Extractions were performed on 520-mg samples of the standard packing contained in cells of two different geometries; one short with 1.0 × 1.0 cm dimensions, and the other long, with 7.3 × 0.37 cm dimensions (length of bed × I.D.). The total volume of the sorbent beds were *ca.* 0.79 cm^3 for both cells, with the short cell having a 1:1 length to diameter ratio of and the long cell a 20:1 length to diameter ratio. Extractions were performed with carbon dioxide at 100.0°C at an average flow-rate of 600 $\mu l\ min^{-1}$, controlled by varying the length of a linear capillary restrictor. Blockages were minimized by heating the length of the fused-silica capillary and agitating the collection vessel in an ultrasonic bath (Branson, Shelton, CT, USA). Each extraction was carried out with the same total volume of carbon dioxide. The extracted compounds were collected by inserting the end of the outlet restrictor into a 10-ml volumetric flask containing several milliliters of absolute ethanol. An amount of 2.5 μg of fluorene was added to the volumetric flask as an external standard. Identification and quantitation of the extracted standard PAHs was performed using a Hewlett-Packard Model 5890 Series II GC and a HP5971 MSD or flame ionization detection.

RESULTS AND DISCUSSION

SFC retention data for the PAHs studies is listed in Table I, along with some physical and spectroscopic properties for these compounds. The widely differing physical and spectroscopic properties of these compounds can make their separation

TABLE I

PHYSICAL, SPECTROSCOPIC AND SFC CHROMATOGRAPHIC PROPERTIES OF PAHs STUDIED

PAH	Formula	Melting point (°C)	Boiling point[a] (°C)	λ_{max} (nm)	k (C_{18})	k[b] (Al)	k[c] (Si)
Naphthalene	$C_{10}H_8$	81	218	275	0.54	0.48	0.23
Anthracene	$C_{14}H_{10}$	217	340	251	1.97	1.50	0.81
Pyrene	$C_{16}H_{10}$	150	404S	240	4.07	2.60	1.35
2,3-Benzanthracene	$C_{18}H_{12}$	347	380S	274	6.26	3.55	1.99
Perylene	$C_{20}H_{12}$	274	340S	252	14.10	5.20	–
Benzo[*ghi*]perylene	$C_{22}H_{11}$	278	> 500	299	26.03	–	–
Coronene	$C_{24}H_{12}$	427	525	301	50.16	10.00	5.71

[a] S = sublimes.

[b] Data from ref. 20.

[c] Data from ref. 21.

and identification difficult. The chromatogram for the isoconfertic (having constant density)–isothermal SFC separation of the PAHs on the C_{18} column, used to generate the *k* value listed in Table I, is shown in Fig. 1. Fig. 1 illustrates the difficulty in achieving complete separation and the difficulty in finding a single UV wavelength that gives sensitive detection of all of the PAHs studied. Although considerable progress has been made in recent years, the relationships between solute properties and their retention in supercritical fluid chromatography is still not well understood. Correlations between various physicochemical parameters of solutes and their chromatographic retention (*e.g.* capacity factor, *k*) may provide valuable insights into the dominant retention mechanisms and may provide useful predictive information for establishing optimum experimental parameters [19]. A variety of physical parameters have been shown to correlate with chromatographic retention. A list of several of these parameters for the PAHs studied is given in Table II.

These parameters are different measures of molecular structure for the PAHs studied. In general, molecular structure can be broken down into three primary elements; namely, the number of atoms, the kinds of atoms, and the linking pattern or bonding scheme of the atoms. Therefore, the more accurately a parameter incorporates these three elements, the more useful the parameter will be in describing molecular structure. Molecular weight and, particularly, Van der Waals volume are good indicators of the size of molecules, but may actually underestimate the bonding scheme and aromaticity contribution to molecular structure. Molecular polarizability and number of fused rings may overestimate the bonding scheme and aromaticity contribution to molecular structure. The correlation factor and molecular connectivity are indexes derived in an attempt to more accurately describe overall molecular structure. The correlation factor, F, is an index empirically derived from log *k* values for PAHs on C_{18} high-performance liquid chromatographic (HPLC) columns calcu-

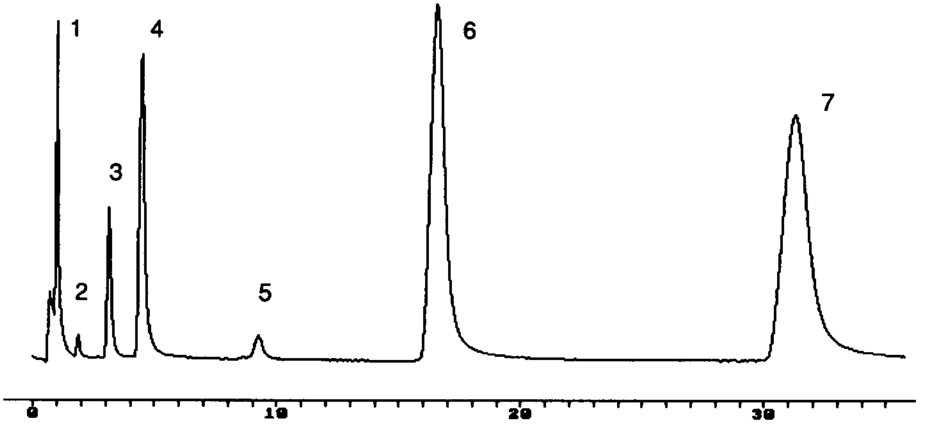

Fig. 1. A chromatogram for an isoconfertic (having constant density)–isothermal SFC separation of 1 ppm mixture of the PAHs on an octadecyl-bonded packed column with supercritical carbon dioxide mobile phase. Column temperature 100°C; UV detector at 290 nm. Peaks: 1 = naphthalene; 2 = anthracene; 3 = pyrene; 4 = 2,3-benzanthracene; 5 = perylene; 6 = benzo[*ghi*]perylene; 7 = coronene.

TABLE II

VARIOUS PHYSICAL PARAMETERS FOR THE POLYCYCLIC AROMATIC HYDROCARBONS STUDIED

PAH	Molecular weight (g)	Fused ring number	Correlation factor (F)	Van der Waals volume ($Å^3$)	Average molecular polarizability (α)	Molecular connectivity (χ)
Naphthalene	128.17	2	5.0	127.1	17.48	3.40
Anthracene	178.23	3	7.0	170.0	25.93	4.81
Pyrene	202.26	4	8.0	185.5	29.34	5.56
2,3-Benzanthracene	228.29	4	9.0	213.9	32.86	6.22
Perylene	252.32	5	10.0	229.0	38.84	6.98
Benzo[*ghi*]perylene	276.34	6	11.0	243.8	41.31	7.72
Coronene	300.36	7	12.0	259.2	42.50	8.46

lated as follows [22]: F = number of double bonds + the number of primary and secondary carbons − 0.5 for a nonaromatic ring.

A more generally applicable index is molecular connectivity. Molecular connectivity has been extensively applied as a structure-based approach to biological quantitative structure–activity analysis and has proven to be a useful tool for establishing relationships between molecular structure and various physicochemical properties [23]. The term molecular connectivity is a descriptive title for indexes from molecular structure [24]. For the PAHs studied, the simplest form of the index can be used. Each carbon atom is designated by a cardinal number called the delta value, δ, which is a count of adjacent or formally bonded carbon atoms. The molecular structure can then be dissected into all constituent bonds formed between carbon atoms i and j. Using the Randic algorithm [25], a value for each bond is computed. The molecular connectivity index, χ, is a simple sum of these bond values over the entire molecule as follows:

$$\chi = \Sigma\,(\delta_i\delta_j)^{-0.5}$$

Correlation between SFC data and various parameters

A linear correlation has previously been reported between the logarithm of SFC capacity factors and fused ring number of PAHs [26]. Correlations between the number of fused rings and SFC retention parameters can only apply to a first approximation, as it is well known that PAHs with the same number of fused rings can have different retention characteristics as seen by the separation of pyrene (peak 3) and 2,3-benzanthracene (peak 4) in Fig. 1. However, fused ring number may be a useful parameter, particularly when comparing PAHs of similar symmetry and those with widely differing number of fused rings. For the present data, we did not observe a linear correlation between log k and fused ring number. In fact, the correlation appears to be logarithmic with fused ring number as seen in Fig. 2 for three different types of SFC systems. The logarithmic curve fit through the data demonstrates the generally good agreement, but also illustrates the limitation in using this parameter, with the data for the four fused ring PAHs, pyrene and 2,3-benzanthracene, lying above and below the fitted line for each plot. This logarithmic relationship is not

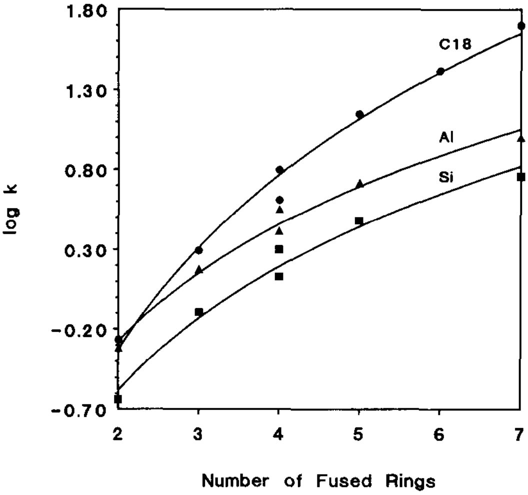

Fig. 2. Plot of log k *vs.* the number of fused rings for PAHs. C_{18} = octadecyl column, 100°C, 300 atm, supercritical carbon dioxide mobile phase; Al = alumina column, 245°C, 47 atm, supercritical isopropanol mobile phase; Si = silica gel column, 260°C, 40 atm, supercritical ethanol–hexane (10:90) mobile phase.

surprising for the alumina and silica gel systems, as it is well known that in adsorption chromatography the following equation is valid [27].

$$\log R^0 = \log V_a + aS^0$$

where R^0 is the specific retention volume, V_a and a are parameters characterizing the adsorbent activity, and S^0 the so-called adsorption energy of the compound. It is surprising, however, to see an apparent logarithmic relationship for the partitioning system, octadecyl column, when linear relationships with physical parameters have been reported previously (*e.g.* 19, 28).

To determine which parameters are most generally useful in predicting SFC retention, we plotted log k *vs.* each parameter, as well as log k *vs.* log (parameter) for the three systems studied. The correlation coefficients for the plots are summarized in Table III in order of best to worst correlation for the parameters studied. For all of the parameters discussed above, with the exception of fused ring number, good linear correlation was seen with the retention data for the octadecyl system. A logarithmic correlation was seen for all of the parameters on the adsorbent systems, alumina and silica gel. Plots with fused ring number and average molecular polarizability, which emphasize the aromaticity of the molecule, generally showed the poorest correlation. Plots with Van der Waals volumes, which emphasize the physical size of the molecule, generally showed better correlation, and molecular weight plots yielded correlation coefficients greater than 0.998. Although the correlation factor demonstrated even better correlation than molecular weight, the parameter which yields near perfect correlation was molecular connectivity (r = 0.9995 to 0.9999), as seen in Fig. 3. As molecular connectivity is relatively simple to calculate for any compound, not just PAHs, this parameter appears to be the most generally useful in describing and predicting retention in SFC.

TABLE III

CORRELATION COEFFICIENTS BETWEEN LOG *k* AND VARIOUS PARAMETERS FOR THREE DIFFERENT SFC SYSTEMS

Parameter	Linear plot Log plot	Column type		
		Octadecyl	Alumina	Silica
Molecular	Linear	0.9995	0.9875	0.9848
connectivity	Log	0.9903	0.9997	0.9999
Correlation	Linear	0.9991	0.9888	0.9865
factor	Log	0.9894	0.9995	0.9999
Molecular	Linear	0.9988	0.9896	0.9876
weight	Log	0.9886	0.9992	0.9998
Van der Waals	Linear	0.9927	0.9920	0.9919
volume	Log	0.9806	0.9954	0.9970
Average	Linear	0.9915	0.9901	0.9953
molecular polarizability	Log	0.9763	0.9928	0.9980
Number of	Linear	0.9872	0.9530	0.9475
fused rings	Log	0.9940	0.9925	0.9889

Correlation between cell geometry and SFE recoveries

To quantify the effect of extraction cell geometry on SFE efficiencies, care was taken to ensure that all of the other controllable experimental variables were kept constant. Temperature, pressure (and, therefore, density), flow-rates, and total volumes were accurately controlled and measured. Extraction conditions were purposely chosen to yield less than quantitative recovery of the PAHs to allow comparisons to

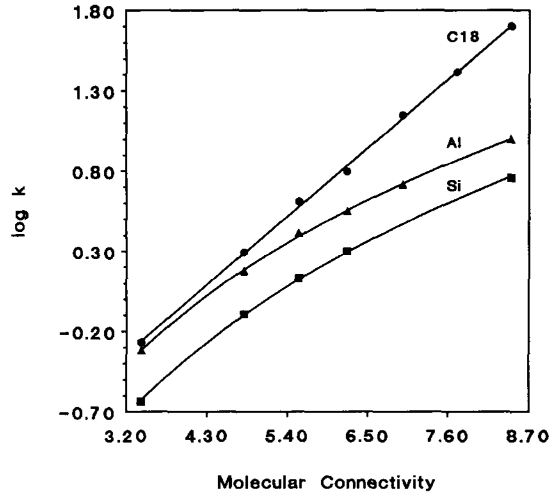

Fig. 3. Plot of log *k* *vs.* the molecular connectivity for PAHs. C_{18} = octadecyl column, 100°C, 300 atm, supercritical carbon dioxide mobile phase; Al = alumina column, 245°C, 47 atm, supercritical isopropanol mobile phase; Si = silica gel column, 260°C, 40 atm, supercritical ethanol–hexane (10:90) mobile phase.

be made between the different extraction cell geometries. Unfortunately, conditions required to yield reproducible recoveries of coronene (> 1%) resulted in quantitative (> 90%) recoveries of naphthalene and anthracene, and, therefore, only the last four PAHs were useful for comparative purposes. The average recoveries for the PAHs from octadecyl packings for two different cell geometries have been reported previously [18]. The average percent recoveries [and standard deviations] for the compounds pyrene, perylene, benzo[*ghi*]perylene, and coronene using the 20:1 (length:I.D.) cell were 60.4 [0.9], 17.1 [0.6], 6.5 [0.7], and 1.7 [0.2], respectively. The average percent recoveries [and standard deviations] for the compounds pyrene, perylene, benzo[*ghi*]perylene, and coronene using the 1:1 (length:I.D.) cell were 80.4 [1.6], 35.8 [0.8], 15.4 [1.4], and 5.2 [0.3], respectively. Each sample was extracted at 100.0°C and 4500 p.s.i. (density = 0.675 ml^{-1}) at an average flow-rate of 600 μl min^{-1}, with a total of 7.5 ml of carbon dioxide.

If the dominant factors which control SFC retention are the same as those controlling SFE, one would expect to see a similar linear correlation between molecular connectivity and the logarithm of the observed recoveries. Fig. 4 shows the plots for the logarithms of the SFC capacity factors and the SFE recoveries for the two different cell geometries studied. Although there is obvious curvature with the SFE recovery data, the linear correlation coefficient appears to improve as the extraction cell geometry is increased (made more column-like). For example, a linear least squares regression analysis for the 1:1 geometry cell yields a $r = 0.973$; whereas, the 20:1 cell geometry yields a $r = 0.985$. Surprisingly, good correlation is seen when the percent recoveries are directly correlated to molecular connectivity, although an opposite effect is seen with cell geometry. As the cell geometry is reduced (made less column-like) the linear correlation increases. For example, a linear least squares regression analysis for plots of percent recovery *versus* molecular connectivity for the

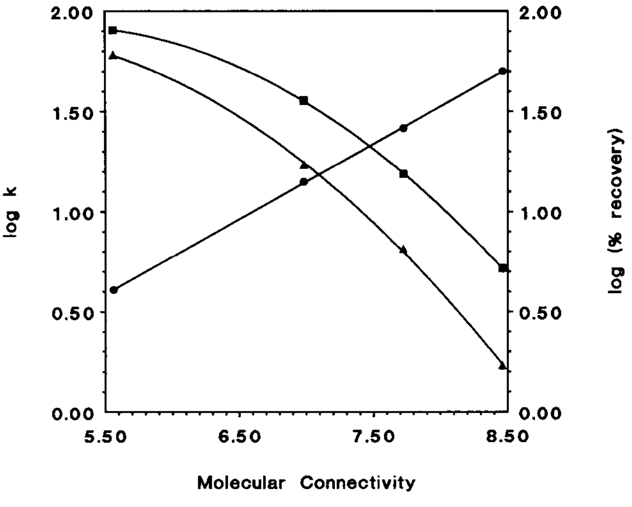

Fig. 4. Plot of log *k* and log (% recovery) *vs.* molecular connectivity for pyrene, perylene, benzo[*ghi*]perylene, and coronene from C_{18} SFC retention data and SFE with different extraction cell geometries. ● = SFC; ■ = SFE; 1:1 cell; ▲ = SFE, 20:1 (length:I.D.) cell.

20:1 geometry cell yields a $r = 0.962$; whereas the 1:1 cell geometry yields a $r = 0.990$. Therefore, it appears that molecular connectivity can be a useful parameter in predicting potential SFE recoveries.

The logarithms of the SFE percent recoveries show excellent linear correlation with fused ring number ($r > 0.996$) for all of the extractions of the present study. Further studies are needed to determine whether there is truly a relationship between these two variables or if this is simply a fortuitous correlation. Unfortunately, the widely differing extractability of these PAHs limits the number which can be compared at a chosen set of extraction conditions, as mentioned previously. Fig. 5 shows the plots of log (% recovery) *vs.* fused ring number for PAHs using the two different cell geometries. It is obvious from this figure that PAHs with a smaller number of fused rings than those studied (*e.g.* 3) are quantitatively recovered; whereas, larger fused ring number PAHs (*e.g.* 8) are generally non-recoverable under the same experimental conditions. For all of the PAHs, a markedly higher recovery was achieved with the short broad cell (1:1) *versus* the long narrow cell (20:1). The relative increase in percent recovery using a 1:1 *vs.* 20:1 extraction cell geometry is 33, 109, 137, and 206% for pyrene, perylene, benzo[*ghi*]perylene, and coronene, respectively.

Correlation between fluid density and SFE recoveries

One of the advantages of supercritical fluids is the ease with which the density, and effectively the solvating power, of the fluid can be changed by varying the pressure at a constant temperature. Selective extractions are possible by performing extractions at successively increasing pressures while keeping the temperature constant. Data for one such extraction is given in Table IV, for successive 3.2-ml supercritical carbon dioxide extractions at 100°C and pressures of 2000 p.s.i. (density = 0.30 g ml^{-1}), 3000 p.s.i. (density = 0.49 g ml^{-1}), 4000 p.s.i. (density = 0.63 g ml^{-1}), and

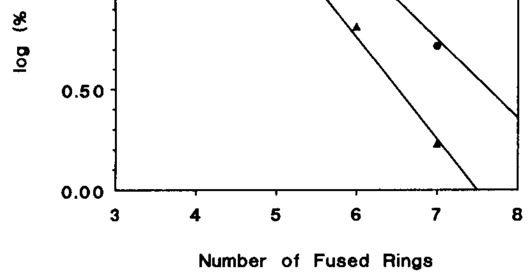

Fig. 5. Plot of log (% recoveries) for supercritical CO_2 extractions *vs.* the number of fused rings for pyrene, perylene, benzo[*ghi*]perylene, and coronene using a 1:1 extraction cell geometry (●) and a 20:1 extraction cell geometry (▲). Extraction conditions: 100°C; 4500 p.s.i. (density = 0.675 g ml^{-1}); flow-rate = 0.6 ml min^{-1}; total volume of carbon dioxide used for each extraction = 7.5 ml.

TABLE IV

RECOVERY (%) OF PAHs FROM AN OCTADECYL-BONDED PACKING AT SUCCESSIVELY INCREASING PRESSURES AT 100°C

Analyte	% Recovery (*ca.* 5% R.S.D.)[a]			
	2000 p.s.i.	3000 p.s.i.	4000 p.s.i.	5000 p.s.i
Naphthalene	61.0	>97	–	–
Anthracene	8.9	42.9	>97	–
Pyrene	0.2	18.0	48.9	>97
Perylene	0.0	7.3	18.3	34.4
Benzo[*ghi*]perylene	0.0	3.2	7.0	13.8
Coronene	0.0	0.0	2.1	4.0

[a] R.S.D. = Relative standard deviation.

5000 p.s.i. (density = 0.71 g ml^{-1}). Again, correlation is observed between the log (% recovery) and fused ring number as seen in excellent linear correlation (r > 0.993) for all of the compounds studied. Linear least squares regression analysis for the data yield the following equations: log (% recovery)$_{\text{pyrene}}$ = 3.281d − 0.321; log (% recovery)$_{\text{perylene}}$ = 3.055d − 0.644; log (% recovery)$_{\text{benzo}[ghi]\text{perylene}}$ = 2.849d − 0.909; log (% recovery)$_{\text{coronene}}$ = 2.737d − 1.347; where d is the density of supercritical carbon dioxide at 100°C. The relative increase in percent recovery upon increasing the supercritical carbon dioxide density 0.12 g ml^{-1} (0.7 *vs.* 0.58) at 100°C is 148, 132, 120, and 113% for pyrene, perylene, benzo[*ghi*]perylene, and coronene, respectively (calculated from the equations above). The average relative increase in recovery, upon increasing the densitiy 0.12 g ml^{-1}, was 128% (standard deviation = 15%), with a

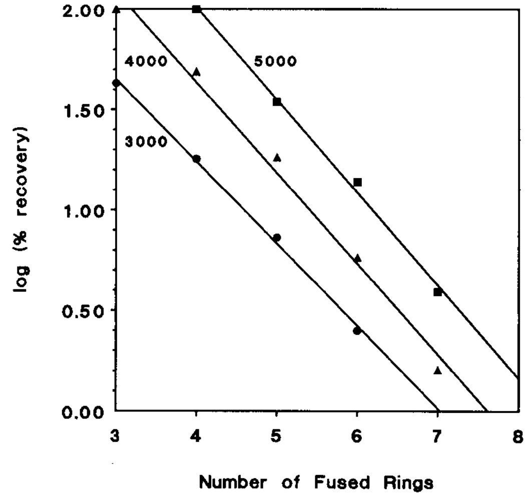

Fig. 6. Plot of log (% recoveries) for supercritical CO_2 extractions at 100°C *vs.* the number of fused rings for pyrene, perylene, benzo[*ghi*]perylene, and coronene at 3000, 4000 and 5000 p.s.i. Extraction conditions: 1:1 cell geometry; 100°C; flow-rate = 0.6 ml min^{-1}; total volume of carbon dioxide used for each extraction = 3.2 ml.

small approximately linear decrease with the number of fused rings for the PAHs studied. This compares to an average relative increase in recovery, upon shortening the extraction vessel from 20:1 to 1:1 (length:I.D.), of 121% (standard deviation = 72%), with a large approximately linear increase with the number of fused rings for the PAHs studied.

The present results indicate that, of the parameters studied, molecular connectivity is the best in evaluating or predicting potential SFC retention characteristics for PAHs. For the partitioning system, octadecyl sorbent, SFC derived log k values directly correlate with molecular connectivity; whereas, for the adsorbent systems, alumina and silica gel, linear correlation is observed with the logarithm of the molecular connectivity. The correlation of molecular connectivity with SFE recoveries of PAHs from octadecyl sorbents is dependent on the extraction cell geometry. For the long narrow extraction vessel (20:1), the molecular connectivity of the PAH shows an approximately linear correlation with the logarithm of the observed recoveries; whereas, for the short broad vessel (1:1), the molecular connectivity correlates directly with the observed recovery. Although supercritical fluid density had the greatest overall effect on achievable SFE recoveries in the present study, it is clear that other variables, including cell geometry, can have effects that are of a similar magnitude and, therefore, must be considered when optimizing a SFE system.

ACKNOWLEDGEMENT

Acknowledgement is made to the FIU College of Arts & Sciences Faculty Development Minigrant Program for support of this research.

REFERENCES

1 J. B. Hannay and J. Hogarth, *Proc. R. Soc. London, A*, 29 (1879) 324.
2 E. Klesper, A. H. Corwin and D. A. Turner, *J. Org. Chem.*, 27 (1962) 700.
3 K. Zosel, *Ger. Pat.* 1 493 190 (1969).
4 B. A. Charpentier and M. R. Sevenants (Editors), *Supercritical Fluid Extraction and Chromatography*, (*ACS Symposium Series*, No. 366), American Chemical Society, Washington, DC, 1987.
5 R. M. Smith (Editor), *Supercritical Fluid Chromatography*, The Royal Society of Chemistry, London, 1988.
6 M. M. Schantz and S. N. Chesler, *J. Chromatogr.*, 363 (1986) 397.
7 S. B. Hawthorne and D. J. Miller, *J. Chromatogr.*, 403 (1987) 63.
8 B. W. Wright, C. W. Wright, R. W. Gale and R. D. Smith, *Anal. Chem.*, 59 (1987) 38.
9 M. E. McNally and J. R. Wheeler, *J. Chromatogr.*, 435 (1988) 63.
10 S. B. Hawthorn, M. S. Krieger and D. J. Miller, *Anal. Chem.*, 61 (1989) 736.
11 J. M. Levy and A. C. Roselli, *Chromatographia*, 28 (1989) 613.
12 K. G. Furton and J. Rein, *Chromatographia*, 31 (1991) in press.
13 K. G. Furton and J. Rein, *Anal. Chim. Acta*, 236 (1990) 99.
14 S. B. Hawthorne, *Anal. Chem.*, 62 (1990) 633A.
15 M. A. McHugh and V. J. Krukonis, *Supercritical Fluid Extraction: Principles and Practice*, Butterworths, Boston, 1986.
16 M. E. P. McNally and J. R. Wheeler, *J. Chromatogr.*, 447 (1988) 53.
17 J. R. Wheeler and M. E. McNally, *J. Chromatogr. Sci.*, 27 (1989) 534.
18 K. G. Furton and J. Rein, *Anal. Chim. Acta*, 248 (1991) in press.
19 K. Jinno, M. Saito, T. Hondo and M. Senda, *Chromatographia*, 21 (1986) 219.
20 S. T. Sie and G. W. A. Rijnders, *Sep. Sci.*, 2 (1967) 755.
21 Y. Hirata, *J. Chromatogr.*, 315 (1984) 39.

22 J. F. Schabron and R. J. Hurtubise, *Anal. Chem.*, 49 (1977) 2253.
23 L. B. Kier and L. H. Hall, *Molecular Connectivity in Structure–Activity Analysis*, Wiley, New York, 1986.
24 L. B. Kier, L. H. Hall, W. J. Murray and M. Randic, *J. Pharm. Sci.*, 65 (1976) 1971.
25 M. Randic, *J. Am. Chem. Soc.*, 97 (1975) 6609.
26 D. Leyendecker, in R. M. Smith (Editor), *Supercritical Fluid Chromatography*, Royal Society of Chemistry, London, 1988, p. 86.
27 L. R. Snyder, *Principles of Adsorption Chromatography*, Marcel Dekker, New York, 1968.
28 K. Jinno and K. Kawasaki, *Chromatographia*, 18 (1984) 103.