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TEMPERATURE AND PRESSURE EFFECTS IN SUPERCRITICAL-FLUID CHROMATOGRAPHY

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SUMMARY

In the search for a suitable programming technique, the effects of pressure and temperature in supercritical-fluid chromatography were studied. Both pressure and inverse temperature programming can be used to influence the retention characteristics. The selectivity of separation can further be controlled by a "moderator". Values of height equivalent to a theoretical plate for model compounds were measured within a wide range of operating conditions and correlated with pressure drop measurements. The results suggest that a compromise between the particle size and pressure drop is necessary.

INTRODUCTION

Chromatography under conditions near the critical point of the mobile phases has gained some attention during the last few years. Several publications[1-10] have dealt with the possibilities of improved separation efficiency and increased migration of larger molecules due to their interactions with supercritical mobile fluids. Experiments have shown that even an imperfection in the carrier gas can cause an appreciable shift of the thermodynamic equilibrium[11,12]. Supercritical-fluid chromatography (SFC) can theoretically offer the following advantages: easier migration of high-boiling solutes and the analyses of thermolabile compounds at lower temperatures; efficiency and analysis time comparable to those obtained in conventional gas chromatography (GC) due to low viscosity and relatively high diffusivity; and the sensitivity of GC.

While the detection problems in SFC remain essentially unsolved, some efficiency and equilibrium studies have been carried out. SIE AND RIJNDERS[13] discussed in some detail the individual factors which may contribute to the values of height equivalent to a theoretical plate (HETP) in high-pressure chromatography. As expected from theory, the longitudinal diffusion in SFC is almost negligible. However, both the resistance to mass transfer and flow non-uniformity contributions play a significant role. The importance of packing techniques was stressed[13] in this type of chromatography.

SFC represents a "bridge" between GC and liquid chromatography (LC) and consequently shares certain features with both methods. Dense gases as well as superheated liquids may be used to enhance the "volatility" of high-boiling solutes. From the instrumental point of view, organic supercritical fluids at moderate pressures[4,5,9]

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are more convenient to use than highly compressed gases[8,10]. While low pressures and pressure gradients are common in GC, the diffusivity and viscosity of liquids demand high inlet pressures and small particles in high-resolution, high-speed LC. As long as favorable flow conditions are met in LC and the geometrical shapes of the particles remain unchanged, there are no limitations other than technical ones for the use of very high inlet pressures and, consequently, high pressure drops. However, the situation is somewhat different in SFC. The effect of pressure drop, which is not directly taken into account in the van Deemter equation, has not been sufficiently stressed. Furthermore, uniform conditions should be carefully maintained along the entire length of the column and unpredictable changes in column pressure conditions limited.

In any type of chromatography, the sample components with different values of partition or adsorption coefficients require programming techniques to carry out their separation under optimum conditions. Temperature and flow programming, techniques of considerable importance in GC, do not generally achieve the same goals in LC. The programmed change in a property of the mobile phase (*e.g.* polarity, pH and ionic strength) has a more pronounced effect on the equilibrium, but the problems of detection and re-use of columns are often realized with solid adsorbents. Extensive comparisons of various programming techniques in LC were made by SNYDER[14]. MAGGS AND YOUNG[15] and SCOTT AND LAWRENCE[16] suggested a combination of temperature programming with a moderator effect in liquid–solid chromatography, but unfortunately long equilibrium times are an apparent disadvantage of this approach.

Specific interactions, which are responsible for an increased migration of the analyzed compounds in SFC, are a very sensitive function of both pressure and temperature. Pressure was therefore suggested by RIJNDERS[17] and JENTOFT AND GOUW[18] as the parameter with a predictable effect from theory. On the other hand, a similar effect can be expected through inverse temperature programming. In studying the analytical aspects of SFC, temperature and pressure were chosen in this work as variables and their effect on the partition coefficients of model compounds and separating systems were investigated within selected intervals in the vicinity of the critical point. Efficiency studies for various compounds under different operating conditions of SFC were also carried out and correlated with pressure drop measurements. The effect of the moderator was also studied. The measurements were compared with those obtained by LC under similar dynamic conditions.

EXPERIMENTAL

The experimental arrangement for SFC and LC was essentially that described by CASHAW *et al.*[10]. A UV monitor (Laboratory Data Control, Danbury, Conn., U.S.A.) was used for the detection of selected compounds. A length of capillary tube placed after the column in the cooling bath converted supercritical fluids back into liquids. The microvalve between the cooling capillary and the detector served to adjust the pressure drop of the chromatographic column. Precision pressure gauges were used for the measurement of column inlet and outlet pressures. Samples were introduced by means of a commercially available sampling valve (Valco Instrument Co., Houston, Texas, U.S.A.).

Redistilled *n*-pentane and isopropanol were used as mobile phases in order to

degas the mobile phase, the temperature of the reservoir was kept slightly above room temperature and a magnetic stirrer used. Columns 1.5 m long with 2.0 and 3.0 mm I.D. were prepared by the conventional dry packing technique.

The dependence of the capacity ratio k (calculated from the retention times of studied solutes and benzene) on pressure and temperature was determined for durene, naphthalene and biphenyl on a 100–120 mesh basic alumina (obtained from Mann Research Laboratories, Inc., New York, N.Y., U.S.A.) column; n-pentane was the mobile phase. 0.1 and 0.5 % solutions of isopropanol in n-pentane were prepared in some cases and their effect on the separation was studied.

Van Deemter curves for benzene and chrysene were measured on the columns packed with spherical materials of different particle size. Corasil I, 37–50 μm, and Porasil, 120–150 and 80–100 mesh (Waters Associates, Framingham, Mass., U.S.A.), were used. The flow rate was measured volumetrically after the UV detector. HETP values for different compounds measured at identical flow rates were also determined.

RESULTS AND DISCUSSION

The thermodynamic reasons for the pressure-induced retention changes have been explained previously[2,3,11,12]. A competitive saturation of the active sites by mobile-phase molecules is an additional factor to be considered in the adsorption type of SFC. RIJNDERS[17] suggested that pressure programming in SFC should have a similar effect to temperature programming in GC. JENTOFT AND GOUW[18] constructed a pressure programmer and used it successfully to separate styrene oligomers up to $n = 32$ in less than 80 min. Our results with aromatic hydrocarbons (Figs. 1 and 2) also indicate that a gradual and predictable decrease of k values with pressure can be achieved at conveniently chosen temperatures. A sudden decrease of k can be noticed in the immediate vicinity of the critical point, resulting in faster elution but also a decreased possibility of separation.
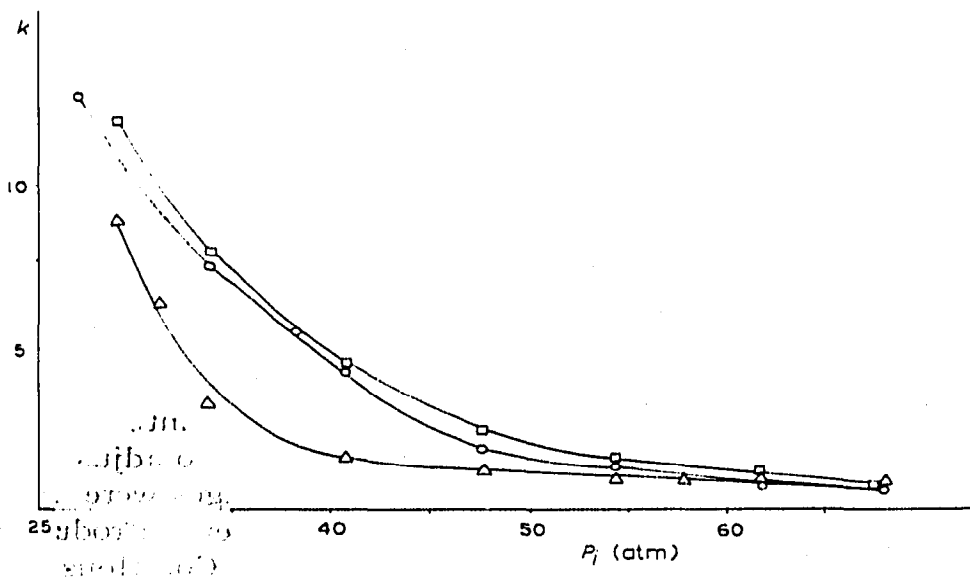

Fig. 1 ... ...nce of k values of biphenyl on pressure at three different tempera¹ ... : △, 198°; O, 21... ...], 228°. Conditions: 1.5 m × 3.0 mm column packed with a 100–... mesh basic alumi¹... ... phase, n-pentane.

When working above the critical pressure, a decrease in the column temperature should theoretically have a similar effect on the distribution coefficients. The effect of temperature on the k values of naphthalene and biphenyl at three different pressures is shown in Fig. 3.

All experimentally obtained curves indicate the presence of a distinct maximum for k. It appears that the position of the maxima depends solely on the mobile phase.
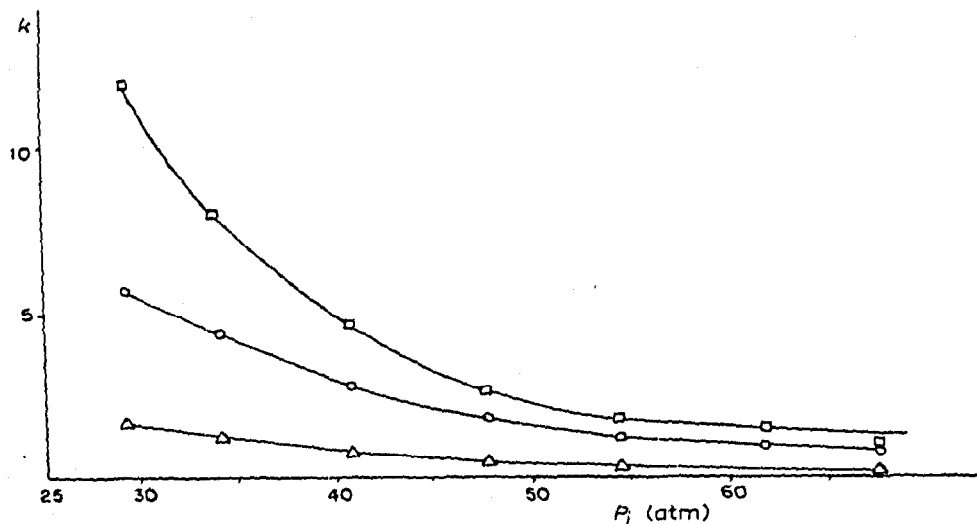

Fig. 2. Dependence of k values of △, durene; ○, naphthalene; and □, biphenyl on pressure at 228°. Conditions as in Fig. 1.
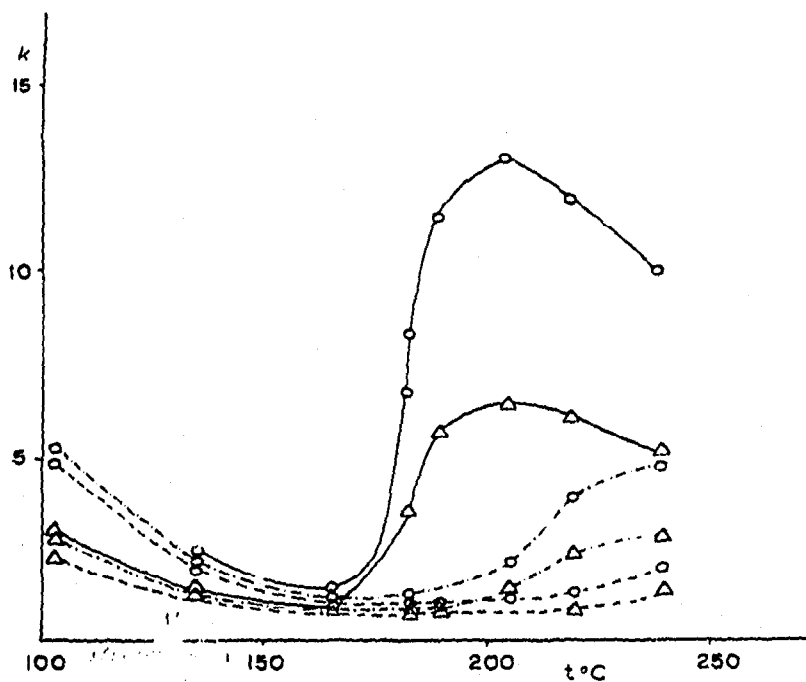
Fig. 3.   ...   ...nce of k values of △, naphthalene and ○, biphenyl on temperatu.. a: three differe... ...   ...s: ————, P_i = 27.2 atm; —·—·—·—, P_i = 40.8 atm; and — — — —, P_i = .+.4 atm. Condi...   Fig. 1.

A change of adsorbent resulted only in different k values, but the general shape of the k vs. temperature curves remained the same.

The migration of solutes in SFC is a very sensitive function of pressure and temperature. From a theoretical point of view, it has a great advantage in regulating the selectivity of the separation process. Indeed, it may obviate the requirement for a large number of suitable fluids and selective packings. From these results, it may be concluded that both pressure and inverse temperature programming can be used in SFC to achieve separations under optimum conditions. Pressure seems to have a more predictable influence. Furthermore, a decrease of flow rate as a consequence of higher density during inverse temperature programming must be taken into account.

Gradient elution techniques are common in LC, and a gradual change in the mobile phase composition can also be used to influence the separation pattern in SFC. The effect of an addition of 0.1 and 0.5 % isopropanol as a "moderator" to n-pentane on k values of different compounds is demonstrated in Fig. 4. A conveniently chosen type of "moderator" may have an important effect. Temperature and pressure conditions can be adjusted so that it will be either present primarily in the mobile phase or covering the active sites of the adsorbent.
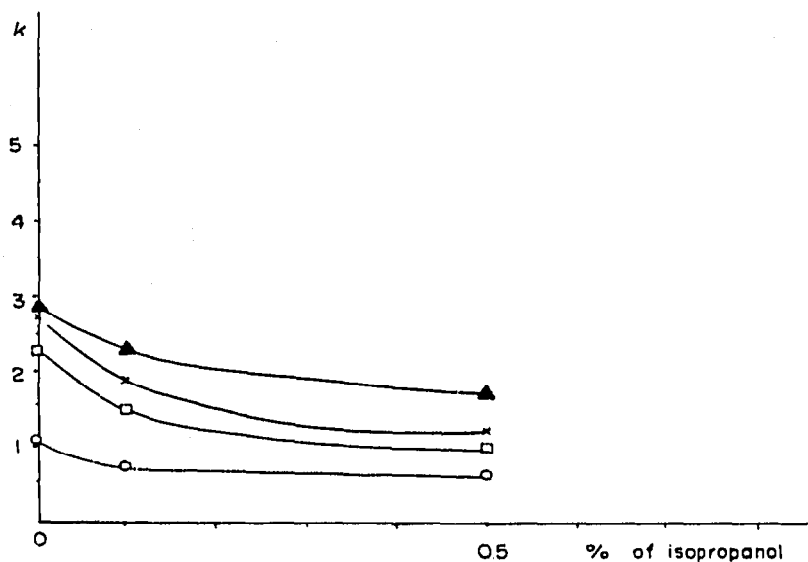

Fig. 4. The "moderator" effect of isopropanol on k values of ▲, chrysene; ○, phenanthrene; ×, benzil; and □, benzophenone. Conditions: 1.5 m × 2.0 mm column packed with a 120–150 mesh Porasil C; mobile phase, n-pentane with an addition of isopropanol.

Further investigations in this study included column efficiency in SFC. If the method is to be of analytical importance, no significant increase of HETP should occur over a wide range of separation conditions. SIE AND RIJNDERS[13] found in their experiments with compressed carbon dioxide that HETP of lower hydrocarbons increased considerably at higher pressures. The C-term of the Van Deemter equation increased with the square of the particle diameter and with solutes of higher molecular weight. It was also suggested that the largest contributors to HETP at high pressures and linear velocities are associated with the non-uniformity of the flow. The importance of a packing technique was stressed and small particles of the size comparable to those used in LC recommended.

The effect of pressure and the pressure gradient in GC has been studied quite extensively (e.g. refs. 20–24). To change the flow rate of a mobile phase in chromatographic columns, the conditions of either constant inlet pressure (P_i) and variable outlet pressure (P_o) or constant P_o and variable P_i are applied. The pressure drop will always correspond to a certain value of flow rate, but the absolute average pressure in the column may be different. NOVÁK and coworkers[22–24] have recently made extensive studies on the performance of gas–liquid chromatographic columns under different pressure conditions and modified the Van Deemter equation to account for the corresponding changes. The conclusions were drawn from both theoretical and experimental evidence that the changes of HETP under different P_i and P_o are simply caused by the changes in the mean absolute column pressure. An increased column length as well as any increase of the column pressure resulted in a slight rise of the C-term and decreased the HETP at lower optimum linear velocities. NOVÁK AND BOČEK[23] also emphasized that such conclusions apply only for strictly laminar flow conditions and moderate pressures.

The situation becomes much more complex in the work with supercritical fluids. Pressure-induced turbulences and the coupling effect[25] are additional factors, which are evidenced by the flattening of the curves of HETP vs. linear velocity. Mass transfer phenomena and non-uniformity of flow are no longer additive factors to HETP and become related to each other under such circumstances.

Since the phenomena involved in SFC seem to be of an extremely complex nature, no attempts were made for their theoretical treatment in this study. Thus, the efficiency studies were essentially made to investigate the merits of SFC as an analytical method. Curves of HETP vs. flow rate for benzene and chrysene as solutes
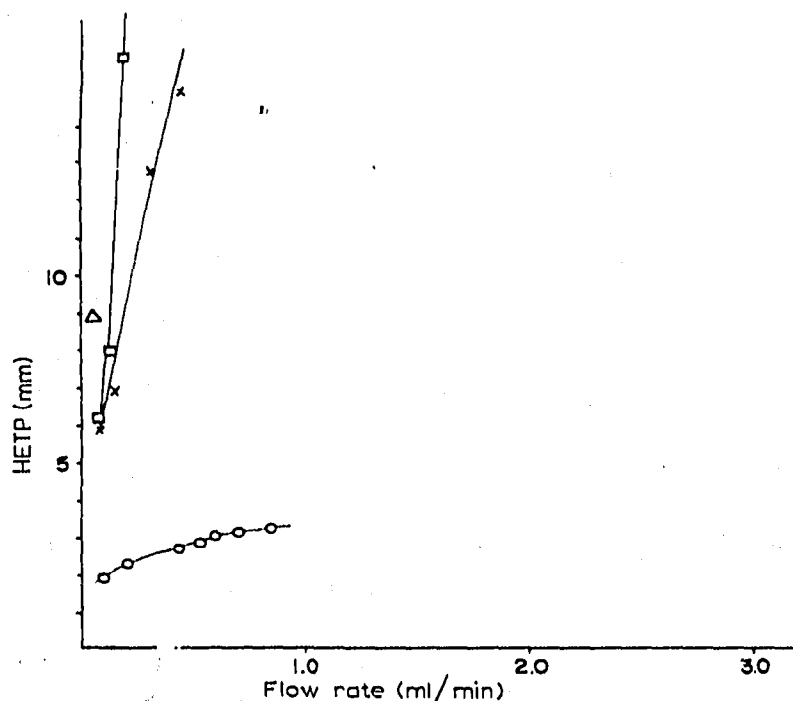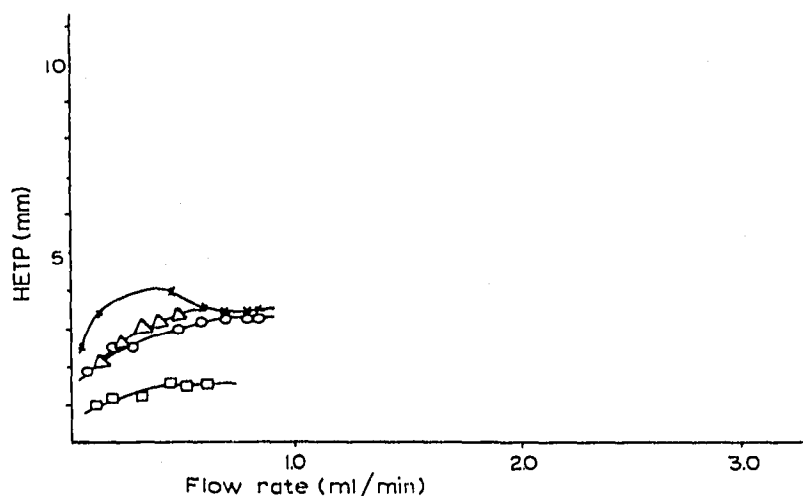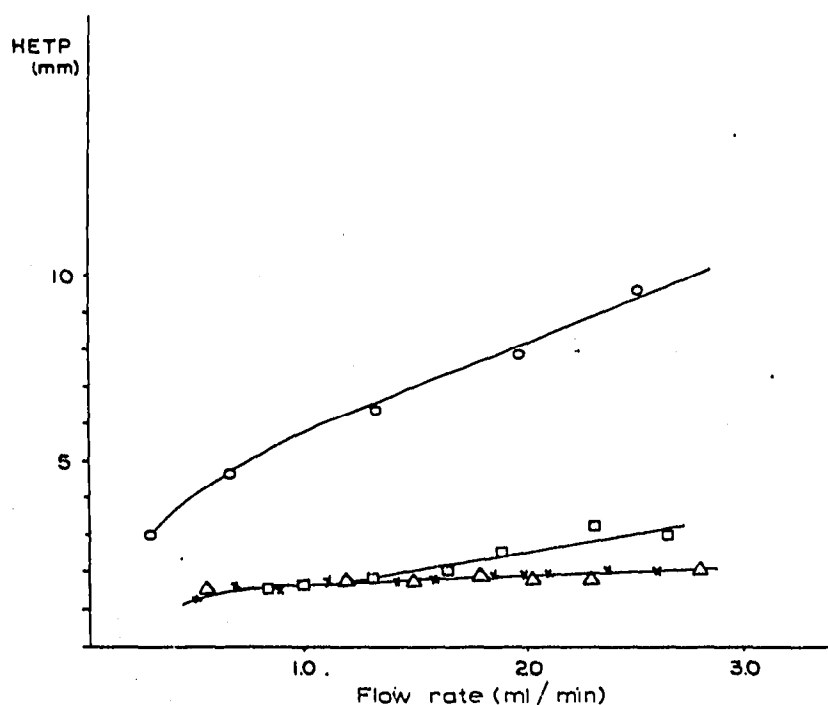

Fig. 5. HETP vs. flow rate curves for benzene under conditions of LC and SFC at different temperatures and pressures: O, room temperature; △, 220° and P_i, 27.2 atm; ×, 220° and P_i, 40.8 atm; □, 185° and P_i, 27.2 atm. Conditions: 1.5 m × 2.0 mm column packed with Corasil I; mobile phase, n-pentane.

under various conditions of LC and SFC are shown in Figs. 5, 6 and 7. While the number of theoretical plates for the benzene peak can be drastically influenced by the operating conditions, the differences for chrysene and other polynuclear aromatic hydrocarbons are quite small. The situation on a 120–150 mesh Porasil column seems to be similar except for the curve measured at room temperature (particle size effect in LC). A considerable flattening of the curves was observed in all measurements with chrysene. Since conditions in the region between laminar and turbulent flow can be

Fig. 6. HETP vs. flow rate curves for chrysene under conditions of LC and SFC at different temperatures and pressures: ○, room temperature; △, 220° and P_l, 27.2 atm; ×, 220° and P_l, 40.8 atm; □, 185° and P_l, 27.2 atm. Conditions as in Fig. 5.

Fig. 7. HETP vs. flow rate curves for chrysene under conditions of LC and SFC at different temperatures and pressures: ○, room temperature; △, 220° and P_l, 27.2 atm; ×, 220° and P_l, 40.8 atm; □, 185° and P_l, 27.2 atm. Conditions: 1.5 m × 2.0 mm column packed with Porasil C (120–150 mesh); mobile phase, n-pentane.

expected, the pressure gradient as a function of flow rate was measured in all cases (see Table I). The results obtained with columns of different particle sizes are quite revealing (Fig. 8). The particle size, which is evidently important in the LC runs, did not seem to have any particular effect in SFC (compare Figs. 8 and 9). High inlet pressures with Corasil are necessary to achieve the separation speed comparable with Porasil columns. Also, the gain in better mass transfer conditions due to smaller particle size might be offset here by less uniformly prepared beds and large pressure

TABLE I

PRESSURE DROP FOR n-PENTANE AS A FUNCTION OF FLOW RATE UNDER VARIOUS CONDITIONS OF LIQUID AND SUPERCRITICAL-FLUID CHROMATOGRAPHY

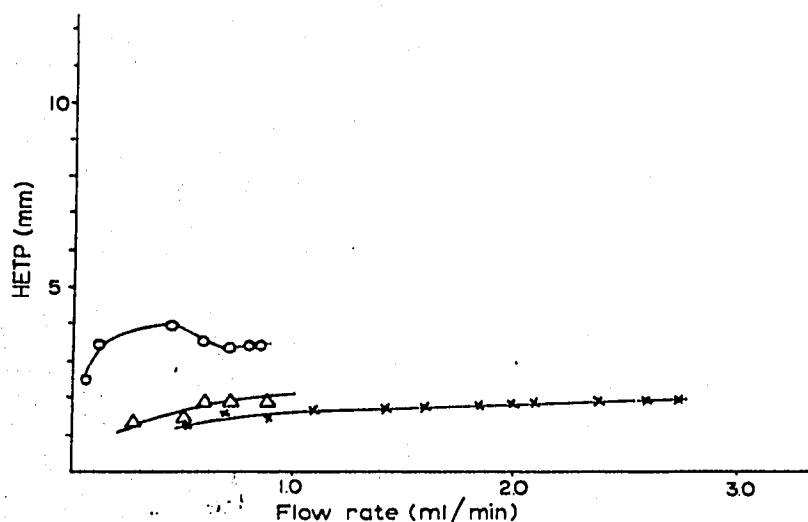| Flow rate (ml/min)	Pressure drop, Δp (atm)			
	Room temperature	$P_i = 27.2$ atm, $t = 185°$	$P_i = 27.2$ atm, $t = 220°$	$P_i = 40.8$ atm, $t = 220°$
(A) 1.5 m × 2 mm column packed with Corasil I (37–50 μm)				
0.1	2.7	1.4	1.0	0.7
0.3	8.8	4.1	4.1	2.4
0.5	14.6	7.8	7.8	5.4
0.7	21.0	13.6	15.6	9.5
0.9	26.8	—	—	13.9
(B) 1.5 m × 2 mm column packed with Porasil C (120–150 mesh)				
0.2	1.3	0.7	0.7	0.7
0.5	2.7	2.0	2.0	1.3
0.8	5.1	4.4	4.4	2.7
1.2	9.5	9.2	10.4	4.8
1.5	13.6	18.0	25.2	7.8
1.8	17.7	—	—	11.6
2.0	20.4	—	—	15.3
2.3	25.2	—	—	25.8

Fig. 8. HETP vs. flow rate curves for chrysene under conditions of SFC at 220° and 40.8 atm obtained with the columns of different particle sizes: ○, Corasil I; △, Porasil C (80–100 mesh); ×, Porasil C (120–150 mesh). Conditions: 1.5 m × 2.0 mm columns; mobile phase, n-pentane.

drops. Similar results were obtained with isopropanol as a mobile phase. A compromise between the particle size and the pressure drop must obviously be made. HETP values for different UV-absorbing compounds measured under identical flow rate, temperature and pressure conditions are listed in Table II. Good efficiency appears to be retained for heavier rather than lighter solutes but the overall differences are quite small. Different molar absorptivity coefficients of the individual compounds at the wavelength used required concentration ranges from 0.05 to 1 μg.
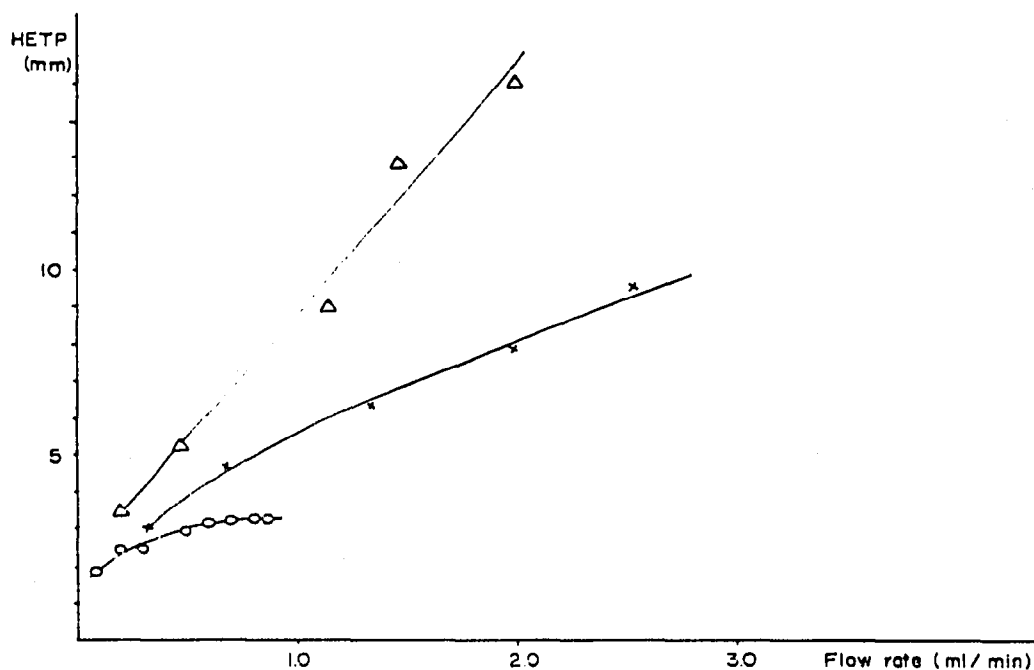
Fig. 9. HETP vs. flow rate curves for chrysene under conditions of LC at room temperature obtained with the columns of different particle sizes: O, Corasil I; △, Porasil C (80–100 mesh); ×, Porasil C (120–150 mesh). Conditions as in Fig. 8.
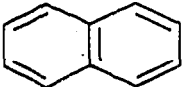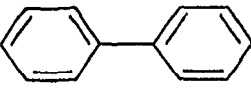
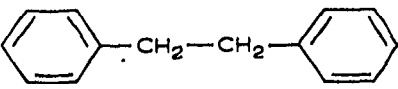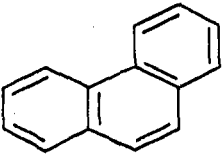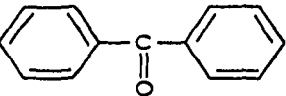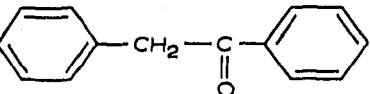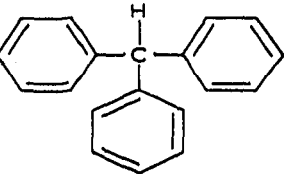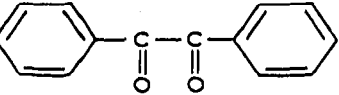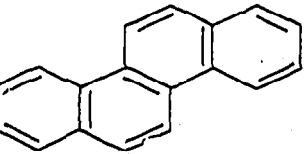Evidence for considerable changes of k values with the flow rate was found in all experiments and it presents one of the most serious drawbacks of this method. Fig. 10 shows k as a function of flow rate for different operating conditions. This is particularly pronounced with the columns of small particle size, greater length and consequently higher pressure drops.

The sensitive changes of solute retention with changes in operating conditions make the analytical application of SFC somewhat difficult. Construction of the system with the required temperature and especially pressure stability is a formidable task with the present state of technology. The cooling capillary, microvalve and pressure gauge at the column exit must significantly affect the efficiency. Introduction of a detection principle which would permit detection of the separated compounds under supercritical conditions and a precise control of the pressure gradient after the detection cell, will be essential to utilize the potential of SFC.

This study has also indicated that ester-type chemically-bonded stationary phases on spherical materials[26] do not possess enough stability under the conditions

TABLE II

HETP MEASUREMENTS FOR VARIOUS COMPOUNDS

Column: 1.5 m × 2 mm Porasil C (120–150 mesh); temp. = 220°; P_i = 40.8 atm; flow rate = 1.0 ml/min.

Solute	Retention time (sec)	HETP (mm)
(benzene)	53	4.5
(naphthalene)	73	3.1
(biphenyl)	84	2.5
—CH₂—CH₂— (1,2-diphenylethane)	96	2.2
(phenanthrene)	117	2.0
—C(=O)— (benzophenone)	188	2.1
—CH₂—C(=O)— (deoxybenzoin)	196	2.2
(triphenylmethane)	163	1.9
—C(=O)—C(=O)— (benzil)	207	2.0
(chrysene)	228	1.7

commonly used. Until more stable packings become available, supercritical-fluid–solid must be preferred over supercritical-fluid–liquid systems.
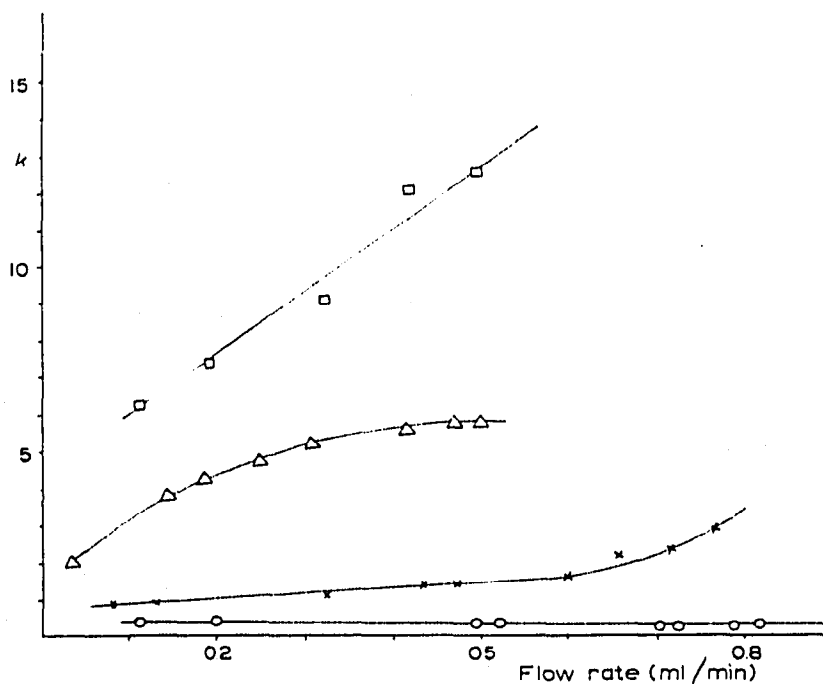

Fig. 10. Dependence of k values on flow rate for chrysene under different temperature and pressure conditions: O, room temperature; ×, 220° and P_l, 40.8 atm; △, 220° and P_l, 27.2 atm; □, 185° and P_l, 27.2 atm. Conditions: 1.5 m × 2.0 mm column packed with Corasil I; mobile phase, n-pentane.

ACKNOWLEDGEMENT

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