Journal of Chromatography, 352 (1986) 315–328
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMSYMP. 671

EFFECT OF PRESSURE ON RETENTION IN SUPERCRITICAL-FLUID CHROMATOGRAPHY WITH PACKED COLUMNS

PETER J. SCHOENMAKERS* and FRANK C. C. J. G. VERHOEVEN
Philips Research Laboratories, P.O. Box 80 000, 5600 JA Eindhoven (The Netherlands)

SUMMARY

An experimental study was conducted on the influence of various parameters on solute retention in supercritical-fluid chromatography, using columns packed with small particles. The influence of the pressure drop over the column on retention was found to be significant. When the column inlet pressure was kept constant, an initial decrease in the capacity factor with increasing pressure drop was observed. A tentative explanation for this unexpected effect can be given if a lack of thermal equilibrium in the column is assumed. Retention data may be extrapolated towards zero pressure drop if the mean column pressure is kept constant in the experiments. A systematic difference was observed between retention data obtained in this study and those in the literature on columns packed with small particles on the one hand, and literature data obtained on columns packed with relatively large particles or open-tubular columns on the other.

INTRODUCTION

In a previous paper[1] we investigated the possibilities of describing the retention process in supercritical-fluid chromatography (SFC) by means of a rigorous thermodynamic model. This model was based on the principle of corresponding states and on the pseudo-critical method used to determine the behaviour of mixtures. The model served two different purposes: (1) to characterize mobile phases and mobile phase mixtures for SFC in terms of their solubility parameters as a function of pressure and temperature and (2) to describe retention in SFC and to predict the variation of retention with changes in pressure or density. The discussion in this paper will be focused entirely on the second aspect of the model.

The initial results obtained from the model were highly encouraging[1]. Experimental literature data[2] on the variation of the retention of naphthalene with pressure with carbon dioxide as the mobile phase could be described with good accuracy. However, despite the initial success of the model, a series of assumptions had to be made and a series of questions were left unanswered. Some of these questions relate to the more fundamental thermodynamic aspects of the model, and we shall not discuss them here. Other questions are concerned more directly with the retention process in SFC. Two important assumptions involve the behaviour of the stationary

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phase. It has been assumed that the stationary phase behaves as an incompressible homogeneous fluid and that the mobile phase does not dissolve in the stationary phase. The first assumption is very rigorous in view of the stationary phases most commonly used: solid adsorbents, polymeric networks with a high degree of cross-linking and chemically bonded monolayers of organic molecules on silica substrates. The second assumption can be modified. Absorption of a constant amount (or, if a mixture is used as the mobile phase, a constant amount of constant composition) into the stationary phase over the range of experimental conditions will not affect the applicability of the model. In other words, the model is applicable if no absorption occurs or in a flat region of the absorption isotherm. However, recent experimental results obtained by Springston and Novotny[3] suggest that even this modified assumption may not be entirely realistic. The effects of the stationary phase are currently under study and will be reported elsewhere[4].

A final assumption made in ref. 1 in order to facilitate the description of retention in SFC by means of a thermodynamic model was that of a negligible pressure drop over the column. In this paper, we describe an experimental study on the effects of a non-zero pressure drop on retention in SFC. The instrumentation used for this study consisted of conventional liquid chromatography (LC) equipment with some minor (but essential) modifications. It has been shown by Gere *et al.*[5] and Lauer *et al.*[6] that this kind of instrumentation allows the use of SFC for fast and reproducible analysis of non-polar or moderately polar components in mixtures. Indeed, this kind of apparatus provides sufficient reliability and ease of operation to be used on a routine basis[7].

From a theoretical point of view, capillary columns are bound to produce more efficient separations[8]. It has recently been demonstrated that capillary columns can also compete with packed columns in terms of speed for simple separations[9]. As the physical properties of supercritical fluids are intermediate between those of gases and liquids[2,6], the optimal diameters of capillary columns and the experimental difficulties may be expected to be intermediate between capillary gas chromatography (GC) (a routine technique) and capillary LC (a technological challenge). Although capillary SFC is definitely feasible[8,9], detection with typical GC detectors such as the flame-ionization detector is not without problems[10–12], and additional difficulties arise from the requirement to split the sample before introduction into the column.

Packed columns do not involve these practical problems. However, they may give more fundamental problems related to the pressure drop over the column. The effects of the pressure drop on band broadening in SFC have been studied by Graham and Rogers[13]. Retention in SFC is a strong function of the mobile phase pressure[1]. Hence, if there is a significant pressure drop over the column, the capacity factor is bound to vary with the position in the column. The observed capacity factor, *i.e.*,

$$k_{obs} = (t_R - t_0)/t_0 \quad (1)$$

where t_R is the solute retention time and t_0 the retention time of an unretained component, will be somewhere in between the capacity factor under column inlet conditions (k_{in}) and the value under the conditions at the column outlet (k_{out}). As the inlet pressure is always higher than the outlet pressure and as k increases with decreasing pressure, the value of k_{out} will be higher than that of k_{in}. Because of the

variation of the capacity factor with pressure, it may also be expected that k will vary with the pressure drop over the column and hence with the flow-rate. The variation of the observed capacity factor with the magnitude of the pressure drop in packed column SFC has so far hardly been studied. The only experimental data available are from Novotny *et al.*[14]. They found that the capacity factor of chrysene increased with increasing column pressure drop when *n*-pentane was used as the mobile phase under SFC conditions (reduced temperature of about 1.05, reduced pressure about 1.2). The value of k increased slowly from about 1 at a pressure drop (Δp) of about 0.5 bar to about 1.5 at $\Delta p = 5$ bar. Subsequently it increased more sharply to about 3 at $\Delta p = 10$ bar. The column used for these experiments was a typical GC column (1.5 m × 2 mm I.D.), packed with particles of Corasil I (silica) with a diameter of 35–50 μm.

Peaden and Lee[15] gave a quantitative description of the effect of the pressure drop on retention. However, they assumed a linear decrease in pressure along the column length (which corresponds to treating the fluid as an incompressible liquid) and a linear variation of density with pressure (which corresponds to the ideal gas law). This combination of approximations makes their results very approximate. An adequate quantitative description should at least involve a reasonable assumption for the equation of state (*i.e.*, the pressure *vs.* density relationship) and should account for the variation of the mobile phase viscosity with pressure. Therefore, pressure correction factors that can be calculated for GC columns at slightly elevated pressures[16] cannot be used in SFC.

Packed column SFC on modified high-performance liquid chromatography (HPLC) equipment[5,6] may greatly aggravate the problems associated with the column pressure drop. Using particles of 10 μm diameter or less and carbon dioxide at elevated pressures, very high pressure drops may occur. The viscosity of carbon dioxide at 400 bar is about 10^{-4} N s m^{-2}, which is about a factor of 5 higher than the viscosity of *n*-pentane under the conditions of Novotny *et al.*'s experiments[17]. In combination with 10-μm particles, which are a factor of 5 smaller, the pressure drop under conditions of equal flow-rates may be increased by a factor of 125 in contemporary experiments in comparison with data reported earlier[14]. For 3-μm particles, this factor may be about 1000. Indeed, the use of column pressure drops exceeding 100 bar over short columns has become common practice[5].

EXPERIMENTAL

The instrument consisted of a PU 4011 HPLC pump, a PU 4030 controller, a PU 4020 variable-wavelength UV detector and a Series 304 GC oven, all from Philips Analytical (Cambridge, U.K.). Samples were introduced with a Rheodyne (Cotati, CA, U.S.A.) Model 7125 injector, equipped with a 10-μl sample loop. This valve was installed in the detector block of the GC oven.

A stainless-steel heat exchanger was mounted directly on the pump heads. A large flow of ethylene glycol, cooled to approximately −20°C, was pumped through the heat exchanger, as well as the carbon dioxide mobile phase (obtained from Philips Gasfabriek, Eindhoven, The Netherlands). The PTFE tubing on the inlet side of the pump was replaced by 1/16-in. stainless-steel capillary tubing to allow the introduction of carbon dioxide at cylinder pressure (approximately 55 bar at room temperature).

The electronics of the pump were slightly modified to allow operation under constant-pressure conditions. The 4030 Controller was used to vary the pressure with a resolution of 1% full-scale. The full pressure scale could be adjusted to be either 20, 40, 100, 200 or 400 bar. Hence, for example, between 100 and 200 bar the pressure could be varied in steps of 2 bar. The 4030 Controller allowed programming of the pressure along the same lines as gradient elution in LC, *i.e.*, up to nine linear, concave and convex segments.

A pulse damper was installed in the GC oven, ahead of the injector in the stream. This also served for thermal equilibration of the mobile phase before it entered the column. The temperature in the oven at the location of the column was carefully measured with a thermometer. The actual temperature turned out to be within 1°C of the set value. Low dead volume flow-through pressure gauges from Chrompack (Middelburg, The Netherlands) were installed just ahead of the injector and just after the detector in the stream.

The detector was modified to permit detection at high pressures. The main characteristics of the flow cell design are thick (6 mm) quartz windows, minimized, pressurized window areas and copper foil spacers. The detection time constant was adapted to the demands of rapid SFC analysis, and could be selected to be 0.1, 0.2 or 0.4 s.

Flow was controlled by using either a Model 26-1721-24-043 back-pressure regulator (Tescom, Minneapolis, MN, U.S.A.)[5] or a standard reducing valve for carbon dioxide (Hoek Loos, Amsterdam, The Netherlands). The former allows much quicker equilibration of the pressure and is therefore recommended for most practical applications. However, it does not yield a stable flow when low flow-rates are used. In that case the reducing valve was preferred. This yielded a much more stable and reproducible, although not completely pulseless, flow. The signal from the detector and the operating pressure, as recorded by the pressure transducer of the pump, were monitored continuously on a Philips Model 8252 dual-pen recorder (Philips E&I, Eindhoven, The Netherlands). Retention data were obtained from measurements at the peak top.

The column used for all experiments in this study was a CP-Spher C_{18} reversed-phase HPLC column (10 cm × 4.6 mm I.D.) from Chrompack with an average particle diameter of 8 μm. We experienced some problems in determining the value of the hold-up time (t_0), which is required for the calculation of the observed capacity factor (eqn. 1). Similar problems have been met by Van Wasen[18]. He could only determine the hold-up time for columns packed with materials with a large surface area by extrapolation of data obtained with columns packed with low surface area materials. We decided to use the elution time of methylene chloride, which was also used as the solvent for the injected samples, as a t_0 marker. This signal can be made visible as a genuine peak at short wavelengths (*e.g.*, 200 nm) or equivalently as a refractive index pulse at longer wavelengths. The centre of this latter signal was found to coincide with the t_0 estimate obtained at lower wavelengths. There is some indication that methylene chloride may not be truly unretained[7]. However, within experimental error, the refractive index pulse of *n*-pentane appeared at the same time as that of methylene chloride. The injection of a series of homologous alkanes[3] did not yield reliable estimates for t_0, as could have been expected from the non-linearity of plots of ln k *vs.* carbon number in SFC[19].

RESULTS AND DISCUSSION

In order to investigate the effect of the pressure drop over the column on the observed capacity factor in SFC, we measured several series of capacity factors at a constant column inlet pressure and a variable pressure at the column outlet. Some typical results obtained at 50°C are shown in Fig. 1, which shows that, as the pressure drop is increased from a very small value, the capacity factor initially decreases, then levels off and finally starts to increase slowly. This is in contrast with expectations and also with the data of Novotny *et al.*[14]. If the column inlet pressure is kept constant, then the effect of increasing the pressure drop (*i.e.*, decreasing the outlet pressure) can only be such as to reduce the average pressure in the column. Given that a decrease in the pressure will always lead to a decrease in the density, and given the smooth, monotonous increase in the capacity factor with decreasing density[1], the capacity factor is expected to increase continuously with increasing pressure drop in Fig. 1. However, the decrease in the capacity factor observed in Fig. 1 is substantial and highly reproducible. For all five solutes, the difference between the lowest value observed for the capacity factor and the value observed at the lowest column pressure drop is about 20%. Our present instrumentation does not allow reliable measurements to be made at even lower flow-rates. However, the capacity factors do seem to increase further when moving towards $\Delta p = 0$.

The observation of decreasing capacity factors with increasing pressure drop is remarkable. To our knowledge, no suggestion of such an effect has appeared in the literature so far. A possible qualitative explanation for the effect might be a lack of thermal equilibrium in the column. In the absence of a mobile phase flow, we can assume effective thermostating of the entire column, *i.e.*, the temperature may be expected to equal the oven temperature at any point in the column. When the flow-

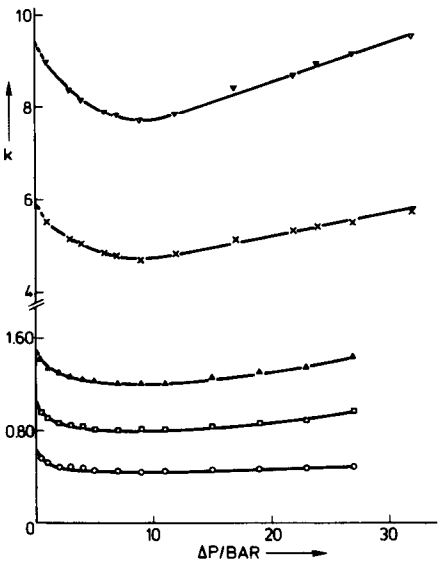


Fig. 1. Variation of the capacity factor with column pressure drop at a constant column inlet of 100 bar. T = 50°C. Solutes: (○) benzene; (□) toluene; (△) ethylbenzene; (×) naphthalene; (▽) biphenyl.

rate increases, two processes will affect the thermal equilibrium within the column: first, heat will be generated due to viscous dissipation, and second, expansion of the mobile phase requires heat from the system. The thermal equilibrium in SFC seems to be more difficult to maintain than it is in either GC or LC. In GC, the pressure drop over the column is usually small. In LC, the pressure drop is large, and hence viscous heat dissipation does occur[20]. However, the solvent is virtually incompressible, and expansion will not be a major factor. It seems possible that in SFC a pseudo-adiabatic expansion of the mobile phase causes a net decrease in temperature inside the column. Under SFC conditions, a decrease in the temperature usually leads to an increase in the density, and hence to a lower value of the capacity factor[1].

We have some further indication of a lack of thermal equilibrium from observations of the peak shape at temperatures slightly above the critical value of 32.8°C. Even at a temperature of 50°C, increasingly poor peak shapes are observed when the pressure drop increases, especially in regions where the capacity factor is a strong function of the pressure (*e.g.*, around 100 bar at 50°C). Even a reproducible peak splitting phenomenon may be observed at high column pressure drops. Finally, opalescence in the detector cell occurs if the column outlet pressure approaches the critical pressure (72.8 bar), even if the oven temperature is well above the critical point.

The strong curvature of the lines in Fig. 1 at low pressure drops makes it very difficult to obtain a reliable extrapolated value for the capacity factor at zero pressure drop. To obtain such values, another means of extrapolation needs to be found. Fig. 2 shows plots of the logarithm of the capacity factor (ln k) *vs.* the pressure drop when the mean column pressure [$\bar{p} = 0.5\ (p_{in} + p_{out})$] is kept constant. It should be noted that this mean column pressure does not equal the average pressure in the column, because of the non-linear variation of pressure along the column. Because the pressure profile over the column is not known exactly, the average column pressure cannot be kept constant. We found that it is sufficient for extrapolation purposes to keep $\bar{p}$ constant. The reason for using ln k instead of k is merely convenience, as this allows a series of curves at different mean pressures to be incorporated in a single figure. Three solutes are shown in this figure: ethylbenzene (Fig. 2a), naphthalene (Fig. 2b) and biphenyl (Fig. 2c). A comparison with the curves in Fig. 1 shows that much less complicated curves are obtained in Fig. 2, where the lines are approximately linear for pressure drops below 5 bar. Fig. 2 was recorded at 50°C, so that the lines at 100 bar may be compared directly with the top three lines in Fig. 1. It appears from Fig. 2 that the variation of retention with pressure drop is small for all solutes at a mean pressure of 80 bar. A strong dependence is observed at 100 bar. The slopes of the lines in Fig. 2 then decrease again at higher pressures, and the lines become virtually horizontal at $\bar{p} = 150$ bar.

Table I gives a quantitative idea of the variation of retention with pressure in the low-pressure range. It can be seen that, to a first approximation, the absolute variation in ln k is independent of the solute, and, hence, that relative changes in k itself will be similar for different solutes.

Fig. 3 shows data recorded for naphthalene at 40°C. It shows that the largest effects of column pressure drop on the capacity factor are now observed at a lower pressure, *i.e.*, around 85 bar. The data at 40°C are entered in the last column of Table I. When these data are compared with those obtained at 50°C, it also appears that the magnitude of the variation is larger at lower temperatures.

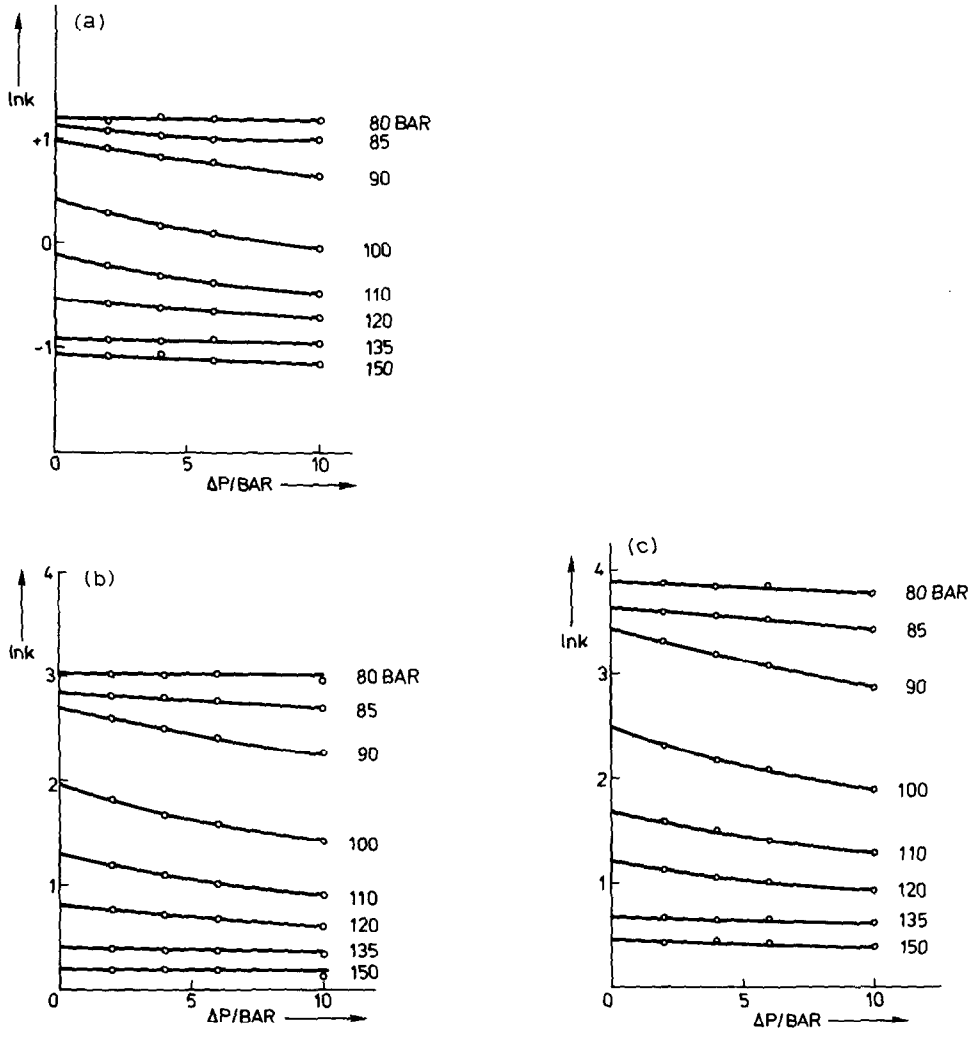


Fig. 2. Variation of the logarithm of the capacity factor with column pressure drop at constant mean column pressure. $T = 50°C$. Solutes: (a) ethylbenzene; (b) naphthalene; (c) biphenyl. $\bar{p} = 0.5\ (p_{in} + p_{out})$.

Fig. 4 shows an alternative presentation of the data for naphthalene. Two sets of curves appear, the set at the top corresponding to an oven temperature of 50°C and the bottom set to 40°C. Each curve corresponds to a given pressure drop over the column. Open circles are actual experimental data points, entered on the figure at the location of the mean column pressure. The closed circles represent the extrapolated value towards a pressure drop of zero. It can be seen that the greatest variations in retention with pressure drop occur under conditions where the variation of retention with pressure is greatest. Hence, if the slope of the retention *vs.* pressure curves is low, for instance at 40°C and 120 bar, then the influence of the pressure drop is understandably low. However, in the range where retention does vary considerably

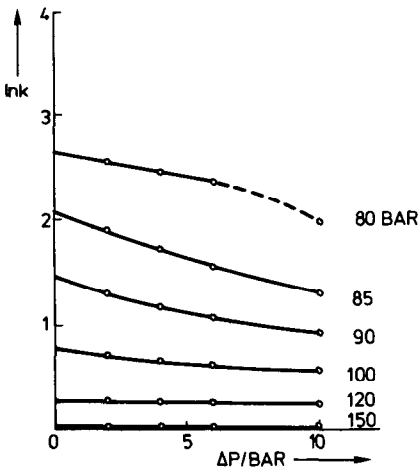
TABLE I

SLOPES OF EXTRAPOLATION LINES IN FIGS. 2 AND 3 ($10^3 d \ln k / d\Delta p$)

$\bar{p}$ (bar)	50°C			40°C: Naphthalene (Fig. 3)
	Ethylbenzene (Fig. 2a)	Naphthalene (Fig. 2b)	Biphenyl (Fig. 2c)	
80	4	7	17	48
85	24	19	25	96
90	41	53	66	65
100	62	70	72	30
110	53	48	48	–
120	21	25	38	2
135	9	7	12	–
150	8	0	4	–

with pressure, as at 40°C and 85 bar, the column pressure drop has a large effect on the observed capacity factors.

Fig. 5 shows the variation of retention (in terms of $\ln k$) with pressure for three solutes at two temperatures. All the data points are values extrapolated towards zero column pressure drop. Characteristic[1] S-shaped curves are obtained for all solutes and, in the pressure range studied, a capacity factor at 40°C is always smaller than that observed at 50°C. The appearance of Fig. 5 suggests agreement between our



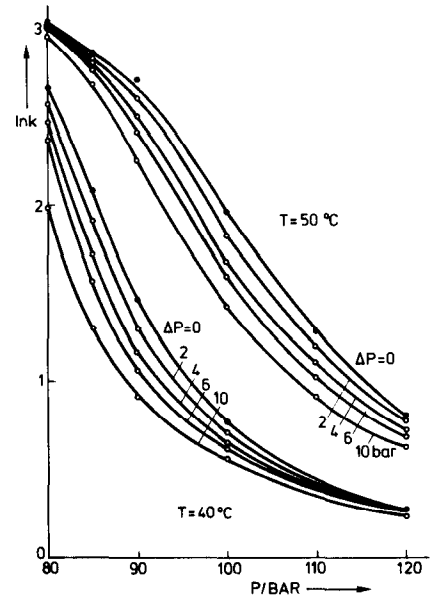


Fig. 3. Variation of the logarithm of the capacity factor with column pressure drop at constant mean column pressure. T = 40°C. Solute: naphthalene.

Fig. 4. Variation of the logarithm of the capacity factor with mean column pressure for naphthalene at 40 and 50°C. Different lines correspond to different column pressure drops. Closed circles represent values extrapolated towards zero pressure drop.

results and literature data[2]. However, a more detailed examination of the data yields a different result. Fig. 6 shows a comparison between the data obtained in this work (open triangles) and those obtained by Van Wasen *et al.*[2,18] (closed triangles) for naphthalene at 50°C. To allow a comparison between the three sets of data, the value for ln k at 100 bar is assumed to be equal to the experimental value observed in this study (1.96). All the experimental capacity factors from Van Wasen *et al.* were multiplied by a constant factor of 7.36, this being the ratio between the observed values at 100 bar. Such a constant factor may be expected to exist between data obtained on different columns when the same mobile and stationary phase are used, because of differences in the phase ratio. After allowing for this constant factor, the different sets of experimental data are expected to be equivalent. Fig. 6 shows that this is not so.

The solid line in Fig. 6 was obtained from the theoretical thermodynamic model[1], which yields the following equation for the variation of retention (ln k) with pressure:

$$\ln k = \ln \varphi_2^{\infty} + \ln (p/\rho) - \frac{p v_2^{L}}{RT} + \ln k_p \qquad (2)$$

where ρ is the density of the mobile phase, which is obtained as a function of p from the equation of state at a given temperature (T), v_2^{L} is the (liquid) molar volume of the solute, R is the gas constant and φ_2^{∞} is the fugacity coefficient of the solute at infinite dilution in the mobile phase. In ref. 1, the pseudo-critical method was used to obtain an estimate for φ_2^{∞}, based on the critical properties of the mobile phase and the solute. Finally, k_p is a constant, which is required because eqn. 2 does not account for interactions in the stationary phase. At a given temperature, eqn. 2 allows the prediction of the complete ln k *vs.* p curve if one experimental data point ("anchor point") is available to calculate k_p. In Fig. 6, the anchor point is taken at 100 bar (ln k = 1.96).

The data of Van Wasen *et al.* appear to agree well with the theoretical model,

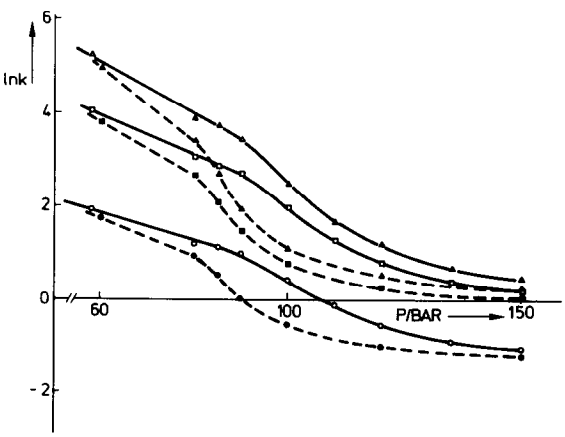


Fig. 5. Variation of extrapolated capacity factors with pressure. Solutes: (●, ○) ethylbenzene; (■, □) naphthalene; (▲, △) biphenyl. Temperatures: 40°C (broken lines; closed symbols) and 50°C (solid lines, open symbols).

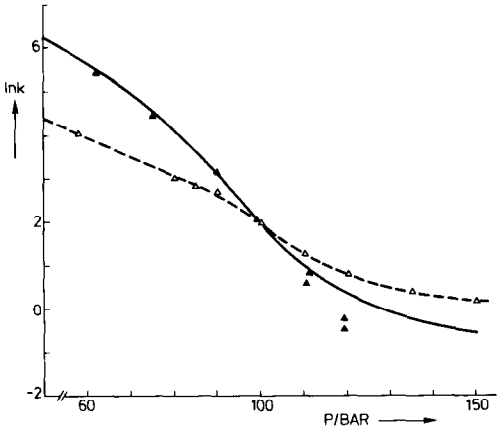


Fig. 6. Comparison between extrapolated experimental data from this study, literature data and a theoretical curve. Solid line, theoretical model[1]; ▲, Van Wasen[18]; △, this study. Solute: naphthalene. T = 50°C.

as discussed before[1]. However, the present data show a much smaller variation of retention with pressure. In fact, if the present data for ln k are plotted against the data of Van Wasen *et al.*, a reasonably straight line is obtained with a slope of about 0.5, rather than the expected slope of 1.

Fig. 7 shows a comparison similar to that shown in Fig. 6, but now at 40°C. Clearly, a similar large discrepancy between the two sets of data is observed.

Fig. 8 compares data obtained in this study at 55°C (open triangles) with data at the same temperature published by Gere *et al.*[5] in the pressure range 100-200 bar. Again, a theoretical line has been drawn. An ln k value of 2.2 at 100 bar is used as the common anchor point for all three data sets. In this instance, we find a closer agreement between the two sets of experimental data, but a systematic deviation from

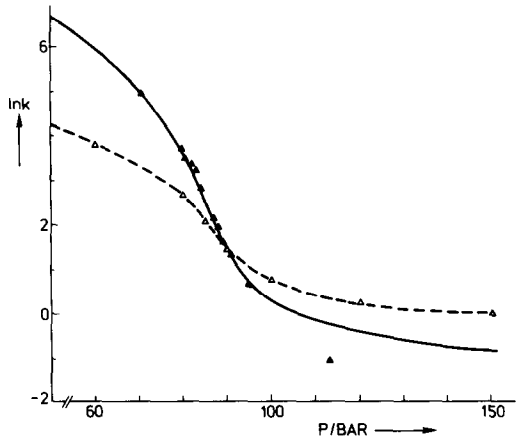


Fig. 7. Comparison between extrapolated experimental data from this study, literature data and a theoretical curve. Solid line, theoretical model[1]; ▲, Van Wasen[18]; △, this study. Solute: naphthalene. T = 40°C.

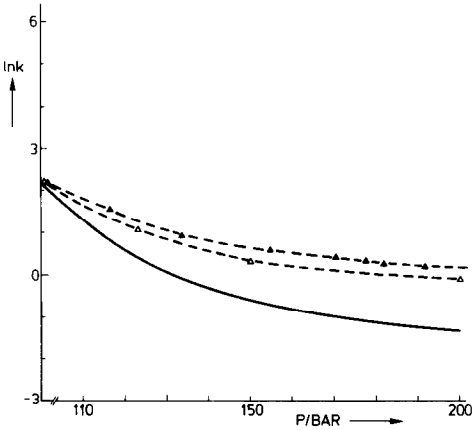


Fig. 8. Comparison between extrapolated experimental data from this study, literature data and a theoretical curve. Solid line, theoretical model[1]; ▲, Gere *et al.*[5]; △, this study. Solute: naphthalene. T = 55°C.

theory. The data of Gere *et al.* show an even smaller variation with pressure than do our data, although the differences between the two data sets are minor. Moreover, care must be taken in comparing the present extrapolated data with the data obtained by Gere *et al.*, who used a large pressure drop over the column.

The experiments performed by Van Wasen *et al.*[2,18], by Gere *et al.*[5] and by us all involved reversed-phase stationary phases and pure carbon dioxide as the mobile phase. Slight differences in the bonded alkyl groups (octyl in ref. 18 *vs.* octadecyl in ref. 5 and in our work) are unlikely to affect the slope and the shape of retention *vs.* pressure plots[1]. An experimental comparison[2] between pure silica and alkyl-modified silica supports this conclusion. One potential source of differences between the three data sets lies in the determination of the hold-up time (t_0). Van Wasen[18] reported considerable difficulties in measuring t_0, and he ended up by extrapolating data obtained from the elution volume of carbon tetrachloride on columns with a low specific surface area in order to calculate the t_0 values for columns with larger surface areas. Details of the procedure followed by Gere *et al.*[5] are not known. However, the differences between the different data sets are so large that gross errors in the t_0 values need to be assumed in order to account for the observed discrepancies. This is all the more true because the differences between the different data sets are not increased at lower k values, where the effect of errors in the determination of t_0 would be much more pronounced (*cf.*, eqn. 1). The differences between the different data sets may only be explained in terms of t_0 if it is assumed that methylene chloride shows a considerable retention at low pressures, while it approaches the true t_0 value when the pressure is increased. Another potential difference between the three sets of experiments is in the kind of particles used to pack the column. Van Wasen *et al.* used relatively large, pellicular particles with a diameter between 37 and 50 μm. In our study, we used particles of 8 μm diameter, while Gere *et al.* probably used even smaller particles. This corresponds to the effects observed in Figs. 6–8, in that the strongest variation of retention with pressure was observed by Van Wasen *et al.*, a much smaller variation by us and an even smaller variation by Gere *et al.* Therefore, this is a vague indication that the particle size in packed column SFC may affect the observed capacity factors.

Of course, a change in particle size will in itself not affect the capacity factor other than by a constant (phase ratio) factor. Hence, if the observed differences between different data sets were indeed connected with the particle diameter, the effect should be an indirect one. In order to alter the variation of the capacity factor with pressure to a great extent, two possible explanations seem possible, as follows.

(a) The thermodynamics of the distribution of the solute over the two chromatographic phases differ considerably. If the effect of the column pressure drop can be neglected (after extrapolation) and if the nature and composition of the mobile and stationary phases are identical in all instances, then such a great variation may only be due to changes in temperature. The temperature is known to have a large effect on the variation of retention with pressure[1,2]. It may be that the lack of thermal equilibrium, suggested before, also has an effect on the curves shown in Fig. 5. However, Fig. 5 shows that "flatter" retention *vs.* pressure curves correspond to higher temperatures, while it was suggested that Fig. 1 could be explained at least qualitatively by assuming lower temperatures due to pseudo-adiabatic expansion of the mobile phase. Moreover, because the curves in Fig. 5 involve values for ln k extrapolated towards zero pressure drop, the effect observed in Fig. 1 is not relevant for the interpretation of Fig. 5. If thermal effects are nevertheless a major factor, then the column diameter would also become a relevant parameter[20]. Van Wasen *et al.* used a column of I.D. 3 mm, whereas both Gere *et al.* and we used columns of I.D. 4.6 mm.

(b) Another way to alter the thermodynamics of the distribution equilibrium is to consider the chromatographic phases to be different in different sets of experiments. As the mobile phase does not change, the only way for such a phenomenon to occur seems to be absorption of mobile phase (carbon dioxide) molecules into the stationary phase. In order to allow for the differences observed in Figs. 6–8, it appears that a large amount of mobile phase would have to enter the stationary phase and, moreover, that this amount would have to be highly dependent on the pressure. The agreement between the data measured by Van Wasen *et al.* and the theoretical model suggests that the effect would also have to be much larger for smaller particles. A possibility might be that at high pressures an effect similar to capillary condensation causes the existence of a liquid carbon dioxide phase within the pores of the packing material or even between the particles.

At present, it is not clear whether either of the two effects described above is indirectly responsible for the apparent effect of the particle size on retention in packed column SFC. We are now studying the effect of particle size more carefully. However, measurements with very small particles under conditions of negligible pressure drop are difficult to perform, owing to the extremely low flow-rates that are required in such instances.

The theoretical model derived in ref. 1 appears to describe the variation of retention with pressure correctly and in quantitative terms when columns packed with relatively large particles are used. Fig. 9 shows an application of the model to data obtained by Springston and Novotny[21] on capillary columns, and indicates that the experimental data obtained for five polyaromatic hydrocarbons are described reasonably well by the model. Some deviations do occur in the region around 50 bar, where retention varies strongly with pressure, but the overall magnitude of the variation is again predicted quantitatively. This is a considerable achievement from the

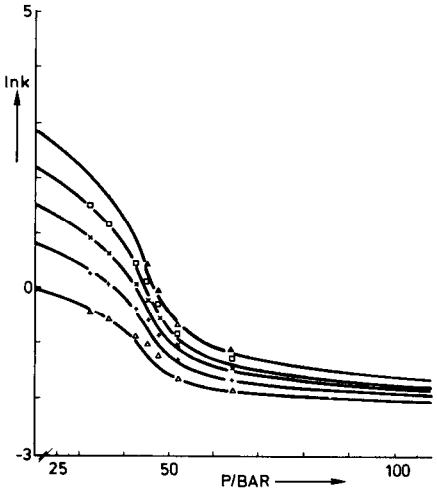


Fig. 9. Comparison between literature data for capacity factors in capillary column SFC[21] and a theoretical model[1] (solid lines). Solutes: (△) naphthalene; (+) phenanthrene; (×) chrysene; (□) picene; (▲) six-ring homologue. Mobile phase, *n*-butane; stationary phase, SE-54; T = 16.8°C.

theoretical point of view, not only because a completely different mobile phase (*n*-butane) was used for the experiments, but also because the critical properties of the polyaromatic hydrocarbons had to be estimated (for details, see ref. 1). Fig. 9 suggests that the theoretical model provides an accurate description of the variation of retention with pressure in both capillary and packed columns with relatively large particles (about 40 μm). For (HPLC-like) columns, packed with very small particles (10 μm or less), there is still a considerable disagreement between theory and experiment.

CONCLUSIONS

The pressure drop over packed columns in SFC has a significant effect on the observed capacity factor, especially in regions where the retention varies considerably with pressure. The relative changes in the capacity factor are independent of the magnitude of the capacity factor and of the solute.

If the column inlet pressure is kept constant, the observed capacity factor initially decreases when the pressure drop is increased. A tentative explanation for this unexpected effect may be given if it is assumed that the actual temperature of the mobile phase in the column is lower than the oven temperature owing to a "pseudo-adiabatic" expansion effect.

A reliable extrapolation of observed capacity factors towards a zero column pressure drop can be made if the logarithm of the capacity factor is plotted against the pressure drop at constant mean column pressure.

The variation of retention with pressure observed on columns packed with small particles (10 μm or less), both in this work and in the literature, is different from that observed for columns packed with relatively large particles (around 40 μm) or for open columns. Therefore, it seems that the particle diameter might have an indirect effect on the fundamental process of retention in SFC.

The variation of retention with pressure in columns packed with relatively large particles or in capillary columns may be predicted from theory.

It has been suggested in this paper that a lack of thermal equilibrium and the use of small particles are further factors that complicate the process of SFC in typical HPLC columns. Both effects may be expected not to play a role in capillary SFC.

The complicating factors mentioned above are not serious obstacles to the application of packed column SFC in practice, especially when pressures far above the critical point are applied.

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