

CHROMSYMP. 128

FUNDAMENTALS OF COLUMN PERFORMANCE IN SUPERCRITICAL FLUID CHROMATOGRAPHY

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SUMMARY

The principles governing chromatography with a supercritical mobile phase are examined in light of measurements made with open-tubular columns. Plate height vs. linear velocity curves obtained for uncoated columns having different radii confirm earlier theoretical predictions. These measurements and their implications for both practical separations and physico-chemical measurements in supercritical fluid chromatography are discussed. Consideration is also given to other parameters such as selection of phase systems, film thickness and operating pressure.

INTRODUCTION

Early attempts to use dense gases and supercritical fluids as chromatographic mobile phases for the efficient separation of thermally labile and non-volatile compounds during the 1960s¹⁻³ were soon overshadowed by high-performance liquid chromatography (HPLC), an instrumentally less complicated technique. While work on supercritical fluid chromatography (SFC) was continued in several laboratories during the 1970s, research in this area was generally considered "esoteric", the instrumentation complex and the scope of applications limited. Recently, interest in SFC has been revived owing to the possibilities of using capillary columns⁴⁻⁶ and small-particle columns⁷ in this mode of chromatography. Not only have the numerous technological limitations of past years been overcome through miniaturized system design, but perhaps more important, the use of supercritical fluids may result in new detection possibilities, including combination of this method with mass spectrometry⁸⁻⁹.

Although it is unlikely that continued development of SFC will be a straightforward task, the course may prove analytically rewarding. One of the major potential achievements of SFC remains enhancement of column separation performance, made possible by relatively fast solute diffusion and low mobile-phase viscosities; the relationship of these quantities to efficient separations was established long ago¹⁰. Both a preliminary assessment and the initial separation results appear to endorse the theoretical potential of SFC.

Initial studies, however, indicate that much remains to be learned about physico-chemical phenomena related to SFC separations. As dense gases and supercritical fluids are rapidly becoming important in a number of scientifically and technologically inter-

esting areas, an improved understanding of processes related to SFC is a worthwhile goal.

In terms of analytical and instrumental goals, certain parallels are apparent between SFC and micro-column HPLC, another developmental trend in several laboratories. While instrumental aspects of SFC in the capillary mode⁶ and in contemporary packed columns⁷ are described elsewhere, this paper will assess the main physical properties that determine the separation performance necessary to resolve complex mixtures of non-volatile compounds.

PRINCIPLES OF SFC

When a mobile phase is heated above its critical temperature and the pressure of the system is raised to values approaching the critical pressure, the mobile phase begins solvating non-volatile molecules and transporting them through the chromatographic column. The "physical proximity" of the mobile-phase molecules to the solute molecules is the primary cause of chromatographic migration in SFC. By varying either the degree of this interaction or the composition of the mobile phase itself, solute migration can be controlled. Different "threshold pressures" at which various molecules commence migrating through the column were observed by Giddings *et al.*¹¹. Consequent upon pressure increases, the density will approach that of a liquid, but certain intimate solute-solvent interactions, known to occur in true liquids, will never be realized¹¹. Thus, SFC is likely to have limitations in the range of both solute molecular weight and polarity, but this range has never been sufficiently determined.

Generally, almost any substance can be converted into a supercritical state, but practical considerations impose limitations on many systems. Systems that necessitate extremely high pressures for adequate solvation [*e.g.*, gases used in gas chromatography (G.C.)] are avoided owing to the obvious technological difficulties, while excessively high temperatures would be detrimental to the chromatography of relatively labile solutes. Finally, the mobile phase "polarity" is also an important consideration. This can be used to control selectivity and is discussed in a later section.

As one of the primary reasons for our involvement with SFC is the resolution of complex, non-volatile mixtures, the kinetic attributes of the method will primarily be emphasized in this paper. "Brute force", *i.e.*, column performance in terms of high plate counts, has previously been stressed¹² as the primary consideration in successful multi-component separations regardless of the nature of the mobile phase. However, we do not want to minimize the potential importance of both the column efficiency *and* stationary-phase selectivity, as they frequently must be combined for difficult separations (*e.g.*, resolution of optical isomers). Alternatively, mobile-phase selectivity effects could also be explored, although their role here appears to be considerably more limited than in HPLC.

CHROMATOGRAPHIC COLUMNS

As with any other chromatographic technique, SFC offers a choice between open-tubular and packed columns. Whenever maximum resolving power is needed in GC, open-tubular (capillary) columns are now invariably chosen. At present, the situation is considerably less clear in HPLC. In spite of certain optimistic predictions¹³ and the concerted efforts of several leading laboratories towards overcoming the problems with extra-column miniaturization, the future of capillary columns in HPLC is uncertain. However, the likelihood of success in SFC is more evident.

An important factor in SFC is the pressure drop across the column. Unlike GC, where the mobile phase has only a minor influence on retention, and liquid chromatography, where the mobile phase is virtually non-compressible, a pressure gradient in SFC can exert adverse effects on the column processes. Because the density of the mobile phase affects both the solute solvation phenomena and diffusion processes within the column, the large density gradients encountered in tightly packed columns can, under certain circumstances, cause a loss of both chromatographic resolution and control of retention. Both effects were observed in our previous work¹⁴. However, owing to their high permeabilities, open tubular columns are ideally suited for SFC. As shown in a recent optimization study⁵, it should be possible to generate efficiencies between 10^5 and 10^7 theoretical plates without exceeding a pressure drop of 10 bar.

It is our current opinion that both open-tubular and packed columns with small particles should be investigated for use in SFC. While capillary columns offer geometrical simplicity for fundamental investigations and analytical predictability, a recent study by Gere *et al.*⁷ showed some possibilities for fast analyses with conventional 3- and 5- μm HPLC columns. It appears that small-bore (200–800 μm I.D.) columns, packed with 3- μm particles, currently under investigation in our laboratory¹⁵ have some potential for high-resolution SFC analysis. However, the resulting pressure drop and its effects on the column processes will always be the price for enhanced resolution by packed columns.

COLUMN PROCESSES

Diffusion processes in either phase can be responsible for band broadening in SFC. To a first approximation, the Golay equation should be applicable to capillary SFC, where the resistance to mass transfer, C_m , in the mobile phase is given as

$$C_m = \frac{1 + 6k + 11k^2}{(1 + k)^2} = \frac{d_c^2}{96D_m} \quad (1)$$

where k is the capacity ratio, d_c is the column and D_m is the solute diffusion coefficient. Our preliminary results with coated capillaries of different radii⁶ have shown that the measured plateheights were several times greater than those predicted theoretically. Current investigations are aimed at determining different contributions to the plateheight for both non-retained and retained solutes. Initial experiments that involved measuring the band dispersion of fluorescent, polycyclic aromatic hydrocarbons in empty fused-silica tubes provided fundamental information on diffusion characteristics. As can be seen in Fig. 1, the plateheight *versus* velocity curves for a non-retained solute in columns of different internal radii can be fitted to theoretical curves by adjusting the diffusion coefficient. The calculated diffusion coefficients are in the region of $10^{-4} \text{ cm}^2 \text{ sec}^{-1}$, justifying the assumptions made earlier in a theoretical treatment of capillary SFC⁵. Measurements with different hydrocarbon mobile phases (C_2 – C_5) have indicated that the diffusion characteristics of the same solute are practically identical so long as reduced conditions are applied; however, the "extraction powers" of these different media may still differ. The diffusion coefficients were significantly greater than those measured in the same hydrocarbons as liquids.

Diffusion coefficients decrease with increasing solute molecular weight (ring number), as shown in Fig. 2; a 2–3-fold decrease in D_m is realized with the addition of two aromatic rings to the molecule. Values of D_m for different ring isomers appear roughly similar.

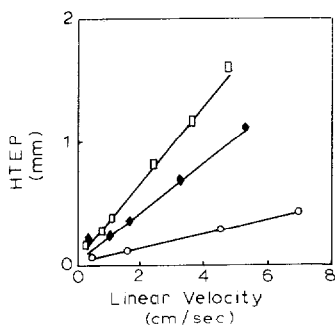


Fig. 1. Efficiency vs. linear velocity for three column radii. Radius: \square , 100 μm ; \blacklozenge , 75 μm ; \circ , 50 μm . Mobile phase: butane, P_r (reduced pressure) = 1.2, T_r (reduced temperature) = 1.03. Sample: anthracene (non retained).

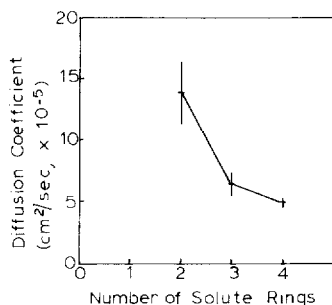


Fig. 2. Diffusion vs. solute size. Mobile phase: butane $P_r=2.0$, $T_r=1.03$. Samples: anthracene, tetracene, picene.

While mobile phase mass transfer in empty tubes appears to be in agreement with the Golay equation, the hypothesis that contributions to the plate height may originate from resistance to mass transfer in the stationary phase was also investigated. It was found that fused silica columns could be coated with successive layers of an immobilized silicone polymer (a methylphenylsiloxane, 50 % aromatic content). A column coated successively with 0.25, 0.50 and 0.75 μm films has revealed certain interesting phenomena. While plotting the plate height as a function of capacity ratio for these film thicknesses (Fig. 3), relatively small differences are observed between different films, suggesting relatively small contributions to the overall plate height from the resistance to mass transfer in the stationary phase, C_s , term

$$C_s = \frac{2}{3} \cdot \frac{k}{(1+k)^2} \cdot \frac{d_f^2}{D_s} \quad (2)$$

where d_f is the film thickness and D_s is the diffusion coefficient in the stationary phase. This is in sharp contrast to the observed behavior of the same column in GC, where thicker films produce visibly inferior results.

Fig. 4 shows a comparison of the plate height *versus* velocity curve for a moderately retained solute (picene, $k = 1.79$) on a coated capillary column with a line predicted by the Golay equation where a previously measured diffusion coefficient and a corresponding capacity ratio value are used. Although the reason(s) for the lower than predicted plate height at high velocities are currently unknown, departures from laminar flow conditions are suspected. The hydrodynamically important ratio of fluid density to viscosity is clearly anomalous for supercritical fluids, possibly causing a relatively early increase in the radial flow component. As theoretically predicted by Tijssen¹⁶ and shown by ourselves with liquids in capillary HPLC¹⁷, making the coil tighter than a certain ratio of coil diameter to column inner diameter will cause a marked decrease in plate height at high velocities owing to "secondary flow". The pertinence of column coil diameter in capillary SFC is currently under investigation. As previously suggested^{18,19}, departures from laminar flow will also have to be considered in work with SFC packed columns.

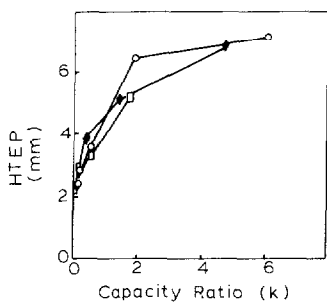


Fig. 3. Efficiency vs. capacity ratio. Mobile phase: propane, $P_r=1.2$, $T_r=1.03$. Stationary phase: polysiloxane. d_f : \square , $0.25 \mu\text{m}$; \blacklozenge , $0.50 \mu\text{m}$; \circ , $0.75 \mu\text{m}$.

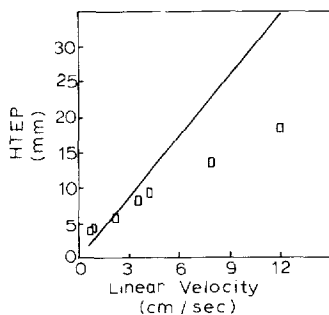


Fig. 4. Efficiency vs. measured linear velocity. Line: Golay equation where $D_m=1.10^{-4}$ $k=1.79$ and $r=107 \mu\text{m}$.

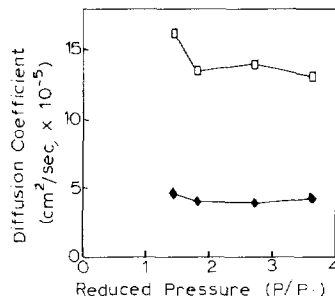


Fig. 5. Diffusion coefficient vs. pressure for anthracene in butane. \square , $T_r=1.03$; \blacklozenge , $T=25^\circ\text{C}$. P_c is the critical pressure.

CONTROL OF SELECTIVITY

Stationary and mobile phase composition and mobile phase density are all factors that affect relative retention. Assuming a constant temperature, an increase in pressure will influence retention according to the following equation:

$$\ln K_p = \ln K_{P_0} + \frac{P-P_0}{RT} (2B_{1,2}-V_2) \quad (3)$$

where K is the partition coefficient, P is the pressure, P_0 is a reference pressure, R is the gas constant, T is the absolute temperature, $B_{1,2}$ is the second virial cross coefficient of the system fluid-solute and V_2 is the partial molar volume of solute in the fluid.

The validity of this relationship is considered to be approximate, as additional virial coefficients are needed at higher pressures. Importantly, an increase in the average column pressure results in enhanced solubilization of increasingly large solute molecules, as the fluid density increases together with intermolecular interactions. A certain degree of selectivity may be imparted owing to pressure-induced equilibrium changes, as has been observed in work with packed-column SFC. Pressure programming in SFC parallels the effectiveness of gradient elution in HPLC and temperature programming in GC.

Mobile-phase density is related to pressure in a fairly predictable way. As shown by Fjeldsted *et al.*²⁰ on a model mixture of polystyrene oligomers, it can be more beneficial to use density gradients rather than pressure gradients to maximize selective interactions and component resolution. Instrumentally, different types of pressure and density gradients can be adjusted through a computer-controlled solvent delivery system. The actual limits of resolution existing in pressure- or density-programmed SFC could have both a thermodynamic and a kinetic cause. In the latter instance, it is interesting to investigate the effects of increased pressure on solute diffusion. Although our knowledge in this area is still far from complete, Fig. 5 suggests that the decrease in the mobile-phase solute diffusion coefficient over an extensive pressure range is small. This is in contrast to the well known solute behavior in the gas phase where diffusivities decrease sharply. The observed behavior in SFC is clearly beneficial in minimizing band

broadening under the density gradient conditions needed to resolve complex mixtures.

As in HPLC, the selection of mobile and stationary phases will greatly influence the relative retention of solutes. Although limited quantitative information exists at present, Giddings *et al.*¹¹ rated numerous different supercritical fluids according to their values of the Hildebrand solubility parameter. Based on this criterion, an equivalent of the eluotropic series can be formed, where a number of attractive phases are encountered. Using a miniaturized SFC system, our choices can easily be expanded to include "exotic" or expensive mobile phases if required for special purposes.

Selectivity effects can further be influenced by using a trace of "moderator" in the mobile phase. This procedure, introduced in SFC long ago¹⁴, has recently been re-emphasized by Gere *et al.*⁷, who discussed possible mechanisms of modifier action.

Finally, selectivity control may also be accomplished through suitable molecular design of the stationary phase. Because immobilized (non-extractable) stationary phases are necessary to prevent removal of the coating by the mobile phase, progress in SFC is greatly dependent on appropriate advances in column technology. Fortunately, several successful approaches to film immobilization have been reported for capillary GC that include both polar and non-polar stationary phases²¹⁻²³. Further general advances in column technology will undoubtedly influence the future applicability of SFC to a variety of analytical problems.

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