Journal of Chromatography
Chromatographic Reviews
Elsevier Publishing Company, Amsterdam
Printed in The Netherlands

SUPERCRITICAL FLUID CHROMATOGRAPHY

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(Received September 27th, 1971)

CONTENTS

1. INTRODUCTION

Earliest man was probably consciously aware of the presence of only the liquid and solid phases of matter. The realization of the existence of a gaseous phase must have come at a much later date. And it was not until in 1869, several hundreds of millenia later, that the existence of an additional state of matter became known. This was when Andrews discovered the critical phenomenon and the supercritical state.

In a sense, there is an analogy between this development and the evolution of column chromatographic techniques. At the turn of the century Tswett, who is credited with the discovery of chromatography, employed a packed bed of granular solids as the stationary phase and used liquid solvents to develop his chromatograms. A few decades later, Martin introduced gas chromatography; but it was only in the early 1960's that the first papers and patents were published on the use of a supercritical fluid as the mobile phase.

In supercritical fluid chromatography (SFC), the mobile phase is, as the name implies, a substance which is maintained at temperatures somewhat above its critical point. Classically, a substance is said to be in the gaseous state when heated to temper-

atures beyond its critical point. However, since the physical properties of a substance in the supercritical state near the critical point are intermediate between those of liquids and gases at ambient conditions, we designate this substance a fluid to distinguish it from a "regular" gas. For chromatographic purposes, such a fluid has more desirable transport properties than a liquid. Hence, SFC is potentially superior to liquid chromatography in separating efficiency and speed. In comparison to a gas, a fluid shows about a 1000-fold increase in solution capabilities. The resultant enhancement in the migration rate of solutes is especially valuable in the analysis of higher-molecular-weight compounds. Another important consideration is that some ionic solutes are soluble in a supercritical fluid. This suggests that SFC may be applicable to the analysis of compounds like the phospholipids[1], which cannot be volatilized (for gas chromatography) without decomposition. There are also other advantages which will be discussed later in this paper.

The number of compounds which can be analyzed by SFC is potentially enormous. Of the 10^6 known compounds which are currently more or less well characterized, only about 15% can be volatilized without decomposition. Most of these compounds are obviously best analyzed by gas chromatography (GC). A substantial portion of the balance, such as proteins, synthetic and natural polymers, lipids, carbohydrates, vitamins, synthetic drugs, metal organic compounds, and many other substances, may well fall in the domain of SFC.

Because the technique is only a few years old, it has not received much attention in the recent chemical literature. Even so one can derive from the few publications the great promise of this technique to make many separations which are not possible by other chromatographic methods. The large potential of this analytical tool can also be deduced from the remarks and discussions at various meetings, where it is evident that many investigators are working in this area. Lest we create the impression that this technique is currently the solution to many problems in analytical separations, we hasten to add that there are still a number of instrumental and operational aspects to be solved before SFC can be compared in ease of operation and in resolving power to GC or to modern high-resolution liquid chromatography. We can, however, expect a number of important developments in this separation technique to be reported in the next few years.

The purpose of this review is to delineate the advantages of this method and discuss some of the problems which are currently encountered. We will especially discuss this technique in relation to the supercritical mobile phase. For this reason, we are limiting our discussion to systems where the operating pressure is less than three to four times the critical pressure of the mobile phase and where the absolute pressure is less than 300–400 atm. This limitation may appear to be somewhat arbitrary, but it is actually based on mechanical and economical considerations.

Many of the phenomena described in this article are also observed when a gas under very high pressures is employed as the mobile phase. This high-pressure GC technique, also known as "dense gas" chromatography, owes its existence to the school of GIDDINGS. This approach, where pressures up to 2000 atm have been used, will not be discussed in much detail because reviews have already been published on this subject[2,3]. In addition, the more sophisticated equipment, its associated problems, and the inherently high cost necessary for safe operation at high pressures are sufficient to put this technique beyond the reach of the average laboratory.

2. THE SUPERCRITICAL MOBILE PHASE

When a liquid and its vapor in equilibrium with each other are heated in a confined space, the intensive properties of the two coexisting phases become increasingly similar until, at the critical temperature, the two phases coalesce into a fluid and acquire the same properties. When this substance is heated beyond the critical temperature, a supercritical phase is obtained; the substance is then called a supercritical fluid. In SFC, the temperature of the mobile phase is generally maintained below 1.2 T_c where T_c is the critical temperature in °K. Operating pressures are, as observed earlier, limited to below three to four times the critical pressure.

For comparative purposes, some physical properties of a gas, a liquid, and a supercritical fluid are shown in Table I. The data only show the order of magnitude. One will note that the viscosity of a supercritical fluid is generally comparable to that of a gas. The diffusivity in a supercritical fluid is between that of a gas and a liquid.

TABLE I

PHYSICAL PROPERTIES OF A GAS, LIQUID, AND SUPERCRITICAL FLUID

Property	*Symbol*	*Units*	*Gas*	*Liquid*	*Supercritical fluid*
Density	ϱ	g/ml	10^{-3}	1	0.3
Diffusivity	D	cm^2/sec	10^{-1}	$5 \cdot 10^{-6}$	10^{-3}
Dynamic viscosity	η	poise (g/cm sec)	10^{-4}	10^{-2}	10^{-4}

(a) Choice of the mobile phase

Any compound which is thermally stable to somewhat beyond its critical point can theoretically be used as the mobile phase. In practice, one may wish to consider the solvent strength, the solvent selectivity, the enhanced chemical reactivity of this fluid under supercritical conditions, the thermal stability of the solute at the necessary operating temperature, and the mechanical problems which are associated with the high critical pressures of some substances. The use of an alcohol as an eluent with silica as the solid substrate, *e.g.*, can result in a partial dehydration of the mobile phase to the corresponding olefin.

Table II lists some possible mobile phases and their critical properties. Freons, ethylene, pentane, hexane, isopropanol and carbon dioxide have been actually used in SFC.

From an instrumental point of view, the ideal fluid should have a low critical temperature, T_c, and a relatively high boiling point at atmospheric pressure, T_b. However, even as early as 1890, these two magnitudes were found to be empirically related by:

$$T_b = 2/3\, T_c \qquad (1)$$

where the temperatures are given on an absolute scale[4]. This relation is also known as Guldberg's Rule. Actually, one can derive from the principle of corresponding states that, at a certain fraction of the critical pressure, T_b is a universal fraction

TABLE II

PROPERTIES OF SOME FLUIDS FOR SFC

	B.p. (°C) at 1 atm	*Vapor pressure at 25°C (atm)*	*Critical properties*		
			T_c (°C)	P_c (atm)	ϱ_c (g/ml)
CO_2	−78.5	63.4	31.3	72.9	0.448
NH_3	−33.4	3.8	132.3	111.3	0.24
H_2O	100	0.03	374.4	226.8	0.344
Methanol	64.7	0.16	240.5	78.9	0.272
Ethanol	78.4	0.07	243.4	63.0	0.276
Isopropanol	82.5	0.05	235.3	47.0	0.273
Ethane	−88	41	32.4	48.3	0.203
n-Propane	−44.5	9.4	96.8	42.0	0.220
n-Butane	− 0.5	2.4	152.0	37.5	0.228
n-Pentane	36.3	0.74	196.6	33.3	0.232
n-Hexane	69.0	0.33	234.2	29.6	0.234
n-Heptane	98.4	0.06	267.0	27.0	0.235
2,3-Dimethylbutane	58.0	0.31	226.8	31.0	0.241
Benzene	80.1	0.13	288.9	48.3	0.302
Dichlorodifluoromethane	−29.8	6.2	111.7	39.4	0.558
Dichlorofluoromethane	8.9	1.7	178.5	51.0	0.522
Trichlorofluoromethane	23.7	1.05	196.6	41.7	0.554
Dichlorotetrafluoroethane	3.5	2.3	146.1	35.5	0.582
Chlorotrifluoromethane	−81.4	35.7	28.8	39.0	0.58
Nitrous oxide	−89	56	36.5	71.4	0.457

of T_c. Since the critical pressure, P_c, of most substances is of the same order of magnitude, deviations from this rule are generally small.

If the mobile phase has a high T_b, the compound will be liquid at ambient pressure. After releasing the pressure, the same detection systems can then be used as those employed in liquid chromatography. This is generally the case when the mobile phase has a $T_c > 190$°C. Some of the best fluids in this group are *n*-pentane, *n*-hexane, and isopropanol.

A low T_c will allow the separations to be carried out at low operating temperatures. This is important if thermally labile compounds are to be chromatographed. A mobile phase with a low T_c is also advantageous in preparative work. The problem in this case is related to the subsequent detection of the eluted compounds because most existing detection systems operate at ambient pressure.

When a low-boiling fluid is used as the eluent, volatilization takes place during the decompression step. Since the effluent is now gaseous under ambient conditions, one might expect to be able to use the regular GC detectors for detection purposes. Higher-molecular-weight solutes will, however, precipitate and/or form large molecular clusters during the decompression process, which makes detection very difficult and erratic. Difficulties can also be expected with lower-molecular-weight solutes if they are ionic in nature.

This problem does not exist if the solute is also gaseous under these conditions[5]. For such a substance, however, GC alone would have sufficed to carry out the desired analysis.

These are only some of the considerations to take into account when selecting the mobile phase. Other criteria can be derived from the following paragraphs, where

the influence of the operating parameters on the properties of the supercritical fluid and on the chromatographic separation process are discussed.

(b) Enhanced chromatographic migration rates

The enhanced chromatographic migration of higher-molecular-weight compounds in a supercritical mobile phase was first reported by KLESPER *et al.*[6] in 1962. The decrease in solute retention times is actually only applicable when comparing this technique to GC, where the limiting parameter in the analysis of high-boiling compounds is related to the low vapor pressure of the solute. The reduction in solute retention times can be substantial.

Fig. 1 shows comparative partition ratios of some polynuclear aromatic hydro-

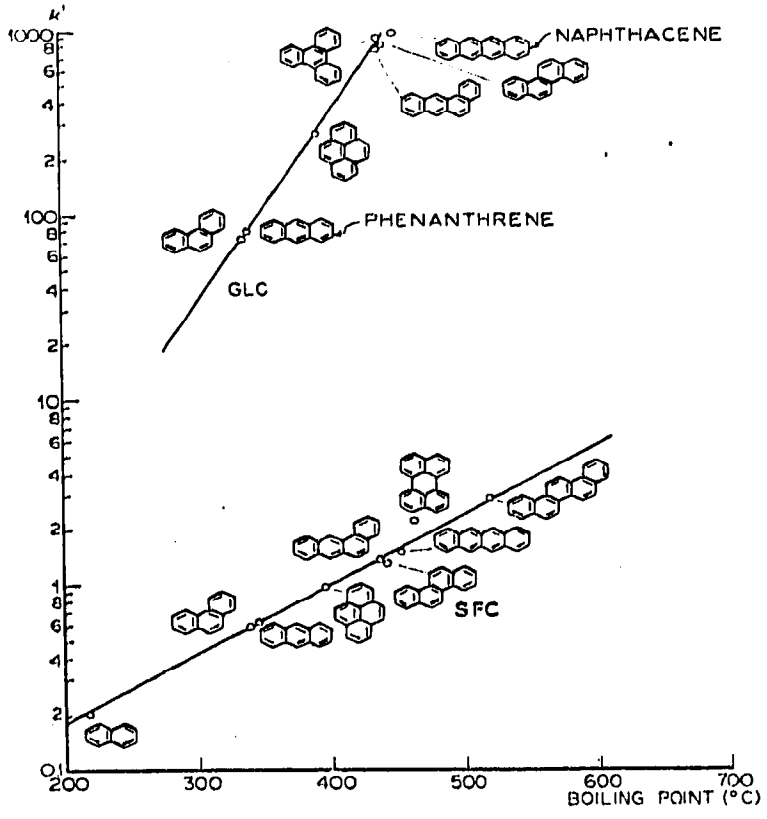


Fig. 1. Comparison of the chromatographic retention of selected polynuclear aromatic hydrocarbons in gas–liquid and in SF–liquid chromatography as a function of the boiling point[7].

carbons in gas–liquid and in fluid–liquid chromatography[7]. These data have been obtained at the same temperatures on comparable columns and equivalent mobile phase velocities. One notes, for instance, that phenanthrene and naphthacene are eluted 120 times and 750 times faster, respectively, in SFC in comparison to analysis by GC.

Accelerated elution times can also be caused by increasing the vapor pressure of the solute. In GC, this can be achieved by increasing the temperature of the column. However, the possibility of thermal decomposition limits the temperature at which a solute can be chromatographed.

Higher apparent solute vapor pressures can also be obtained by increasing the density of the carrier gas. An increase in molecular interactions in the now non-ideal gas phase, and a resultant increase of solute "solubilities" in the carrier fluid are observed. This solubility is at first approximation proportional to the density of the mobile phase. In chromatographic terms, this behavior is equivalent to a sharp decrease in partition ratios or a sharp increase in capacity factors.

For a gas to attain densities and solvent properties of the same orders of magnitude as those of liquids, pressures in the order of 1000 atm have to be used. By using a supercritical fluid, it is possible to obtain high "gas" densities at much lower pressures. In the region of the critical point, there is a sharp increase in density during the transition from gas to supercritical fluid. This change can be as much as a factor of 100 or more during this transition process. A sharp increase in solubility is, therefore, also observed as the temperature and pressure of the solvent approach and exceed the critical conditions[8]. The solubility, which is a function of the density of the system, is not discontinuous at the critical point of the solvent.

The phenomenon of increased solubility in a supercritical phase has already been known for many decades. HANNAY AND HOGARTH made their first observations only a few years after Andrews discovered the critical phenomenon[9]. In their studies on the solubilities of cobalt and ferric chlorides in alcohol, they observed that these compounds dissolved in the supercritical alcohol in much higher concentrations than would have been predicted from the molecular vapor pressure of the salts alone. Other investigators have since then shown in many examples the sharp increase of solubilities in gaseous solvents with increasing temperature and pressure up to the critical point of those solvents.

(c) Pressure

Operating pressures are determined by the critical pressure of the mobile phase and by the required pressure drop for a minimum fluid velocity through the column. Operating pressures in SFC rarely exceed three to four times the critical pressure.

Table II shows that most of the applicable mobile phases have critical pressures below 50 atm. Since pressure drop requirements are minimal in this technique, a practical range in SFC would therefore be around 20–200 atm. This range is comparable to current usage of modern high-resolution liquid chromatography[10]. Even at this moderate range of pressures, many investigators are somewhat concerned over the dangers involved. However, with the current technological knowledge available to generate and contain high pressures, ordinary safety precautions are generally adequate to perform SFC without undue hazards. Fittings, valves, and tubings rated up to 5000 p.s.i. are now readily available as stock items in many commercial inventories.

The use of very high pressures may also be deleterious to the separation process from another point of view. SIE *et al.*[11] and SIE AND RIJNDERS[7] find that with increasing pressure the height equivalent to a theoretical plate (HETP) curve as a function of the flow velocity becomes much steeper, with a concurrent shift of the minimum of this curve to lower flow velocities. This rapid increase in the HETP is mainly determined by the mobile phase mass transfer resistance in the intraparticle pores. This phenomenon is especially noticeable during the transition from gas to supercritical

fluid. In the supercritical region itself, however, the increase in HETP with increase in pressure may be a minor factor in the overall separation process.

Because of the very low viscosity of the fluid, pressure drop requirements are usually not very large. A difference of a few atmospheres or even less is generally adequate to result in the desired mobile phase velocities. For a linear velocity of 3.5 cm/sec through a 400-cm long column packed with particles having a particle diameter, d_p, of 1.2×10^{-2} cm and a shape factor, Φ, of 0.70 (the shape factor of a particle is unity for spheres and decreases with increasing deviations from a perfect sphere[12]), the calculated pressure drop is only 0.3 atm[13]. In actual practice, much higher pressure drops are obviously necessary to drive the fluid through other restricting features in the flow system. For a 180 cm $\times$ 4.5 mm I.D. column, packed with 100–200 mesh alumina and pentane flow rates of 3 ml/min, CASHAW[14] measured an average pressure drop of 20% over the column for column inlet pressures of 40–77 atm (T_c of pentane = 33 atm).

We have mentioned earlier that, in the region close to the critical point, partition coefficients are highly pressure-dependent. One of the most important operating options of this technique is based on this property. This is the use of pressure programming to process mixtures with a wide range of partition ratios. By increasing the pressure, both the flow rate and the solute "volatility" will be enhanced. Very high-molecular-weight compounds can thus be chromatographed in relatively short times without the loss of resolution for the earlier eluting components. By operating isothermally during the pressure-programming step, the relative change in partition coefficients as a function of the pressure is about the same for different compounds of a given molecular type. There is no "cross-over" of the partition coefficients, such as is sometimes observed when temperature-programming is used in SFC. The importance of pressure-programming in SFC can be compared to that of temperature-programming in GC and to solvent-programming in liquid chromatography. It is, therefore, an almost indispensable accessory for operational versatility.

The marked dependence of the partition coefficients on the pressure does, however, preclude the use of high-pressure-drop columns. In these columns, partition ratios will increase appreciably as the solute migrates down the column. This process is equivalent to "holding back" the most rapidly eluting components in the sample. This phenomenon is detrimental to the separation efficiency and decreases the resolution. In extreme cases, this behavior is sufficient to yield totally unusable chromatograms. Under those conditions where a substantial pressure drop is expected, one could operate at two to three times the critical pressure.

Fig. 2 shows the density of propene as a function of pressure and temperature close to the critical point. This diagram is representative of the substances usually considered for a supercritical mobile phase. In the region of two to three times the critical pressure, the change in density with pressure is not very pronounced any more. Another alternative to decrease $\partial\varrho/\partial P$ would be to consider an isotherm at a somewhat more elevated temperature.

Many high-molecular-weight compounds remain essentially stationary on the column until a certain pressure is reached. Migration is then observed as this pressure is exceeded. GIDDINGS[2] calls this the threshold pressure, P_t. This value will obviously vary quite markedly with the fluid employed. Table III lists P_t of some compounds with CO_2 (T_c = 31.3°C) at 40°C as the carrier fluid.

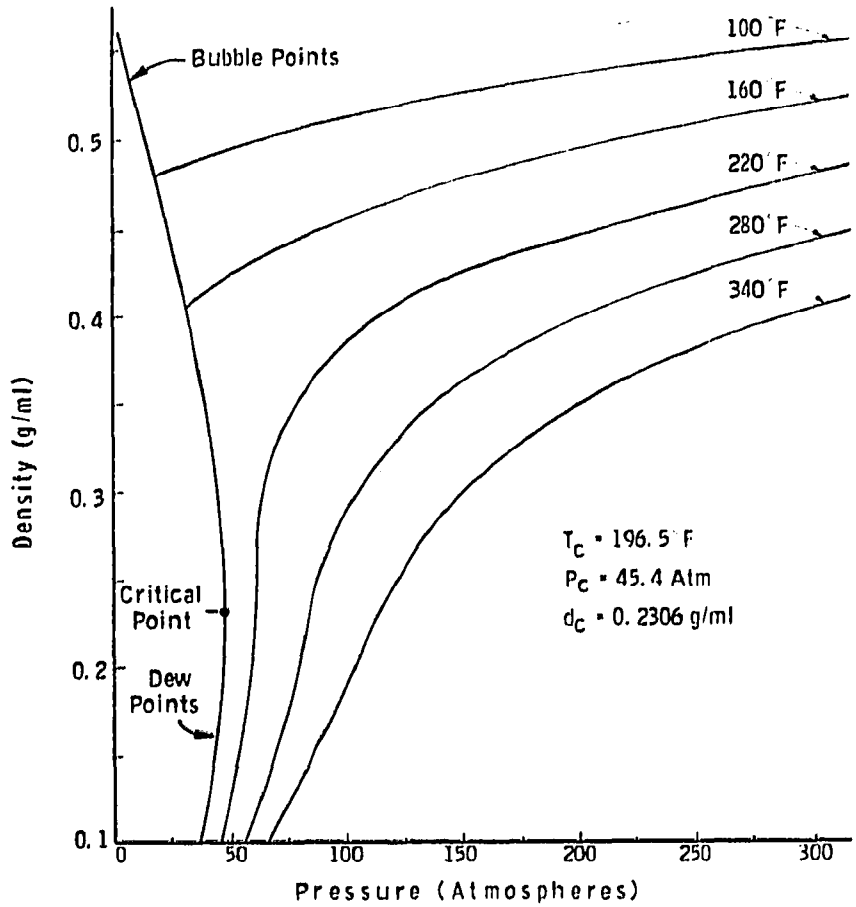


Fig. 2. Density of propene as a function of pressure and temperature close to the critical point.

TABLE III

THRESHOLD VALUES FOR SOME COMPOUNDS WITH CO_2 AT 40°C (ref. 29)

Class	*Mol. wt.*	P_t *(atm)*
n-Hydrocarbons		
$C_{18}H_{38}$	254.5	87.5
$C_{22}H_{46}$	310.5	89.8
$C_{36}H_{74}$	507.0	104.8
Alcohols		
$C_{11}H_{23}OH$	172.3	61.2
$C_{12}H_{25}OH$	186.3	78.2
$C_{18}H_{37}OH$	270.5	98.7
Silicone gum rubber		
SE-30		770
Carbowax		
400	400	90
20,000	20,000	190
Dinonyl phthalate	418.6	165
Apiezon L		820

(d) Temperature

The effect of temperature on the partition coefficient is not as clear and direct

as in GC. Close to the critical point, at a pressure somewhat above P_c, an increase in temperature can result in a sharp decrease in fluid density and hence in solute "volatilities". This phenomenon can be derived from Fig. 2. In some cases, a few degrees difference has been observed to result in a change of the partition coefficients by a factor of two or more. This effect is, therefore, the opposite of what is observed in GC. By increasing the temperature further, partition coefficients can increase, remain approximately constant, or even decrease depending on the solute and on the system. Under isobaric conditions, the lines denoting the partition coefficients as a function of the temperature may intersect each other, even for compounds from a single molecular type[15].

Temperature can, therefore, be used to increase the resolution between any two components; it can also have a deleterious effect on the separation. The applicability of temperature-programming has to be determined separately for each case. In common situations, it is best to operate under isothermal conditions. There is sufficient evidence that in many cases improved chromatograms can be obtained by applying negative temperature-programming instead of increasing the temperature during the chromatographic analysis.

(c) Selectivity

An important criterion to consider is the selectivity of the mobile phase. In GC, separations are carried out basically according to molecular weight. In fluid chromatography, this trend is much less discernible. By using a non-polar mobile phase, conditions become favorable for separation according to group types. The type selectivity which can be obtained is excellent. SIE *et al.*[11] showed that, with *n*-pentane as the mobile phase on alumina, phenylnaphthalene elutes at approximately the same time as hexacosylnaphthalene, which has twenty additional methylene units. Under comparable operating conditions, the highly paraffinic di-*n*-tricontyl phthalate is eluted well before the diphenyl phthalate, notwithstanding the fact that the former compound has 48 more carbon atoms than the latter[15]. In this separation, *n*-pentane was used as the mobile fluid; the stationary substrate was 23% polyethylene glycol 6000 on 120–140 mesh Sil-O-Cel.

If separations are to be carried out according to molecular weight, a polar mobile phase such as isopropanol should be used[5,15]. This approach compensates for the inherent type-selectivity of the method. The observed elution times are then proportional to the molecular weight of the solutes.

The selective behavior is also enhanced by a judicious selection of the liquid substrate. In fluid–liquid chromatography, a polar substrate will enhance group–type separations, while a non-polar substrate should be used if separations are to be carried out according to molecular weight[5,15].

The importance of selecting a mobile phase with the proper strength and selectivity has already been adequately recognized by prominent workers in the field of liquid chromatography. The preceding paragraphs show that this concept is also very important in SFC. Unfortunately, too little is yet known about the intermolecular forces in a supercritical fluid to derive adequate semiquantitative equations to describe this solution behavior in this medium. Adjustment of the solvent strength by varying the mobile phase composition may appear to be not as important in SFC

as in liquid chromatography because the density of a supercritical fluid and, hence, the solvent strength are quite dependent on the operating pressure. Practical considerations make it also less convenient to change the mobile phase composition during operation. However, because of mechanical considerations, pressure alone is insufficient to cover a wide range of solvent strengths. Different mobile phases will have to be used to process compounds with a wide range of solubilities. This concept is already evident from the earliest observations on this technique. KLESPER *et al.* observed that some porphyrins can be chromatographed with dichlorodifluoromethane or monochlorotrifluoromethane but show negligible migration in lower chlorinated Freons, even at much higher pressures[6].

3. COLUMN PARAMETERS

(a) Flow rate, particle size, and column efficiency

The optimum flow rate, v_{opt}, corresponding to the minimum in the HETP–v curve for a chromatographic system can be approximated from GIDDINGS' observation[16] that

$$v_{opt} \sim D_m/d_p \qquad (2)$$

where D_m is the diffusivity of the solute in the mobile phase and d_p is the particle diameter.

For a D_m of 10^{-3} cm^2/sec and an average d_p of 100 μ, the optimum flow rate is around 0.1 cm/sec. This value is about 10^2 smaller than that observed in GC and about 10^2 larger than that computed for a liquid chromatographic system. Since the viscosity of a fluid is of the same magnitude as that of a gas, the pressure drop for the same "reduced" velocities[16] and particle diameters is about 10^2 lower than that observed in a GC column. One would, therefore, tend to operate at much higher flow velocities and somewhat higher HETP's and compensate by using longer columns to achieve a net gain in separation speed.

Practical operating flow rates range from 0.2–10 cm/sec or about 0.5–20 ml/min for a 3-mm I.D. column. Under these conditions, SIE AND RIJNDERS find that the major factors influencing the column efficiency are related to the packing uniformity, intraparticle mobile phase diffusion and the solute diffusivity in the stationary phase[17]. Packing inhomogeneities lead to variations in mobile phase path lengths and velocities. The resultant effect on the column efficiency is called the eddy diffusivity and is equivalent to the "A" term in the Van Deemter equation.

In GC under normal operating conditions, this "A" term is negligible. In SFC, on the other hand, this term can be quite large. For a 6-mm I.D. column packed with 250-μ particles at pressures around 50 kg/cm^2, "A" is approx. 2 mm. This contribution to the plate height is independent of the flow rate and can be reduced by decreasing the column diameter and the particle size, d_p.

The intraparticle mobile phase diffusion contribution to the plate height increases proportionally with the mobile phase velocity. At high velocities, this term becomes the determining factor in the efficiency of the column. This contribution is also proportional to d_p^2. To maintain high efficiency, it is necessary to use very small particles when high fluid velocities are being used.

Fig. 3 shows the effect of the particle size on the plate height, H, for CO_2 as the

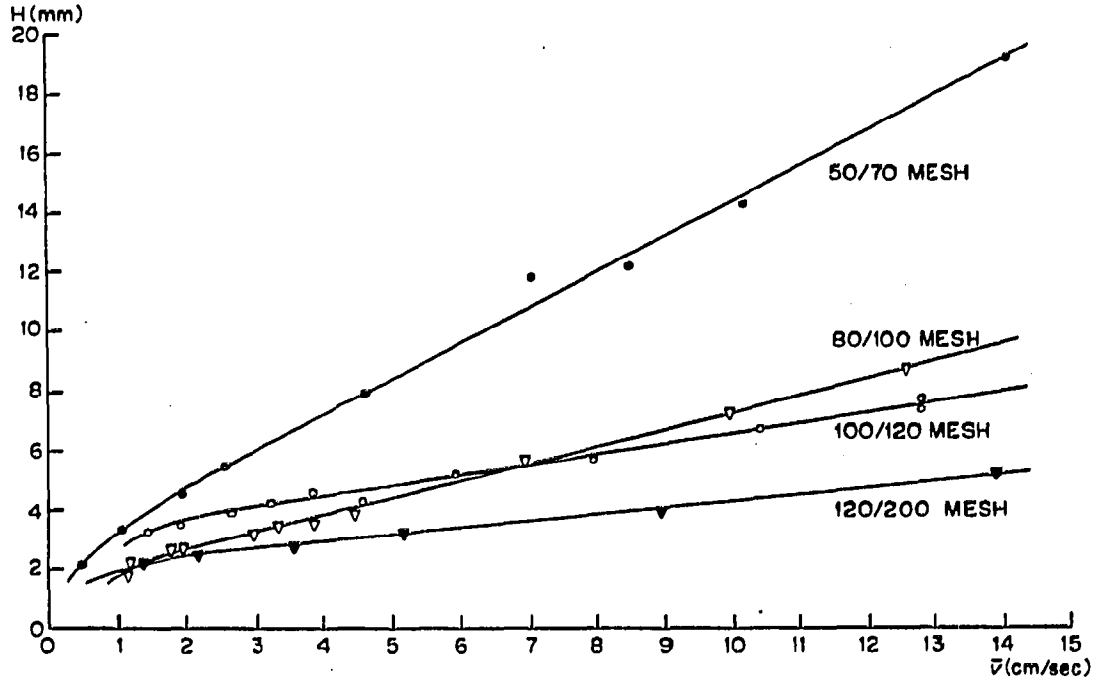


Fig. 3. Plate height as a function of the particle size distribution of the packing and linear flow velocity. The mobile phase is CO_2; the solute is *n*-pentane[17].

mobile fluid and *n*-pentane as the solute[17]. Although the absolute values of H are also dependent on both the solute and the mobile phase, these graphs clearly show the necessity of using small particles if high separating speeds are to be attained.

The disadvantage under these operating conditions is that much higher pressure drops are necessary. The pressure drop is approximately proportional to $1/d_p^2$. We have already mentioned the increase in partition ratios with pressure drop as the solute travels down the column. At high pressure drops, this phenomenon can substantially decrease the attained resolution and make the chromatogram unusable. A possible solution suggested earlier is to operate close to the critical temperatures but at about two to three times the critical pressure where $\partial\varrho/\partial P$ is smaller.

High pressure drops will also be observed with poorly packed columns or columns packed with irregularly shaped particles with a wide range of diameters. In SFC, it is particularly essential to use a very narrow mesh range packing. With the regular packings available commercially, it is important to remove the fines because these small particles contribute very strongly to the pressure drop. The best method to remove fines is by elutriation with a liquid of relatively high density, such as carbon tetrachloride or water[18].

In general, however, we recommend the use of the specialty packings, which are now commercially available. These are the porous silica beads[19], the porous layer beads[20], also called controlled surface porosity packing[21] or pellicular sorbents[22], and the surface-textured beads with little internal porosity[23]. Controlled surface porosity packings are now also available with an organic phase chemically bonded to the surface of the particle[24].

These hard, spherical particles pack uniformly without fracture. The higher degree of shape regularity allows for the preparation of more efficient and reproducible

columns which have very low pressure drops. Bed porosities generally range around 0.40. One disadvantage of this type of packing is its much lower capacity. Much lower sample sizes must, therefore, be used to avoid overloading the column.

An important aspect of the use of the controlled surface porosity packing or the pellicular sorbent is the possibility of a substantial reduction in the intraparticle mobile phase contribution to the plate height without having to decrease the particle diameter, d_p. Decreasing d_p can result, as indicated earlier, in unacceptable pressure drops over the column. In using regular adsorbents as the column packing, the film thickness of the stationary phase, d_f, is equivalent to d_p, since the porosity of the adsorbent allows the solute to diffuse through the whole particle. A reduction of d_f has been attained in these packings without a concurrent reduction of d_p by depositing a thin porous layer of the adsorbent on a fluid-impermeable glass sphere. These beads typically show a d_f of 0.03–0.1 d_p. Since at high fluid velocities the intraparticle resistance to mass transfer is the major contributor to the HETP, the use of these supports allows for a substantial increase in mobile phase velocities without a concurrent, sharp decrease in separation efficiencies.

An additional approach to high separating speeds is to increase the flow velocity to induce turbulence in the column. Because of the low viscosity of a fluid, turbulence should occur at much lower flow velocities than in liquid chromatography. Under turbulent conditions, the enhanced mass transfer in the mobile phase and the coupling phenomenon[16] should both contribute to reduce the plate height at high mobile phase velocities. Studies with gases passed under high pressures through a solid-coated capillary showed that with Reynolds numbers over 2000 a sharp drop in plate height is observed[3]. Because of the very high pressures involved, this aspect will not be further considered in this review.

(b) Stationary phase

The column packings which are used in SFC are essentially the same as those used in high-resolution liquid chromatography. One can, therefore, also differentiate between fluid–liquid and fluid–solid chromatography. In fluid–liquid chromatography, very high-molecular-weight liquid substrates have to be used because of the high solution capabilities of the supercritical fluid. In this respect, the problem is analogous to that observed in liquid chromatography. KARAYANNIS *et al.* observed that Ucon 50HB2000 and Apiezon M were almost completely eluted from the column in a few minutes with dichlorodifluoromethane as the supercritical mobile phase[25]. Silicone gum rubber SE-52 bled continuously, and Harflex 370 was partially decomposed. They found Epon 1001, XE-60 and Versamid 900 especially suitable for the analysis of porphyrins.

In SFC, the stationary liquid phase can also be stripped off by the mobile phase although the loss in efficiency is probably not as critical as in high-pressure liquid chromatography. This problem can sometimes be solved by the use of thermally stable "brush" packings[24,26]. Both CASHAW *et al.*[1] and JENTOFT AND GOUW[27] used Durapak types *n*-octane/Porasil C and Carbowax/Porasil C in their work. Many of the present commercially available "brush" packings are, however, not sufficiently stable for prolonged operation with SFC at temperatures above 150°F.

Under certain conditions, induced condensation of the mobile phase must take

place on the solid substrate with a concurrent increase in the partition coefficients. This condensation of the mobile phase in the capillaries of a porous support or dissolution in the liquid substrate is probably always present to some degree. In the first case, this would lead to a novel kind of fluid–liquid partition chromatography where partitioning would take place between two similar substances. In this form of chromatography, bleeding of the liquid substrate or removal of the bonded hydrocarbons will have little effect on the separation process.

4. INSTRUMENTATION

The instrumentation in SFC is quite similar to that of modern high-resolution liquid chromatography. A number of minor modifications are necessary to make a liquid chromatograph suitable for operation with a supercritical fluid. Fig. 4 shows a schematic diagram of a supercritical fluid chromatograph. One can observe that most of the components are the same as those used in modern high-pressure, high-resolution liquid chromatography.

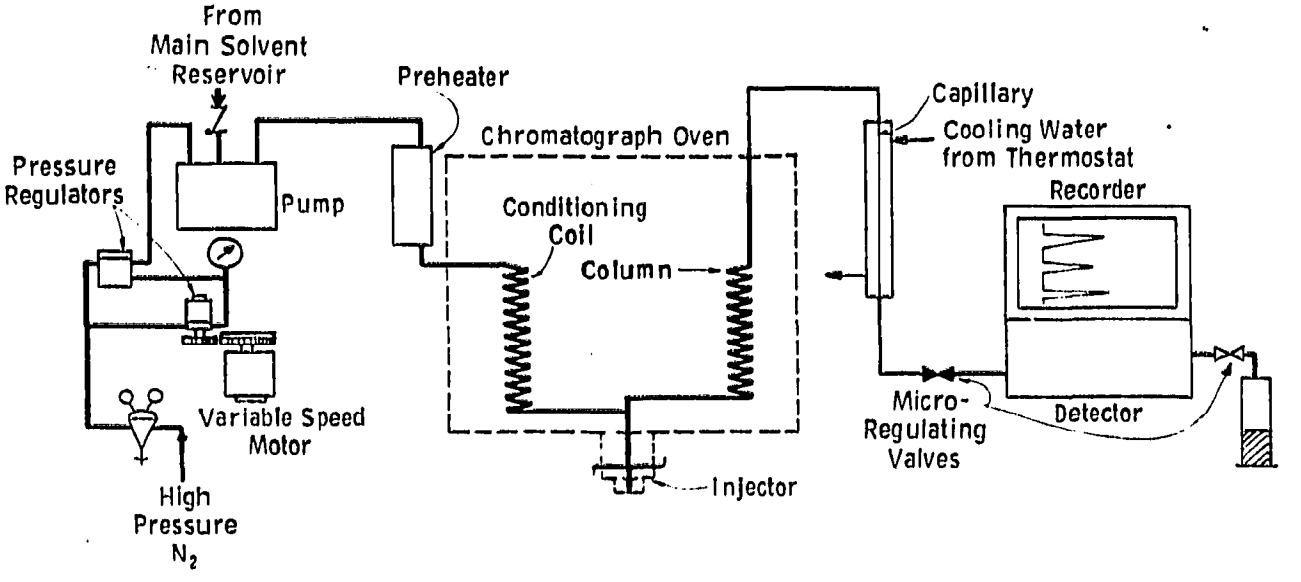


Fig. 4. Schematic diagram of an apparatus for SFC.

(a) Injector

The much greater solvent properties of the mobile phase and the high pressures involved pose many problems to the injector systems used in this technique. Injector systems commonly used in GC fail rapidly if used in SFC. In practice, use is made of special high-pressure injector devices[15,27], some of which already have found prior application in high-pressure liquid chromatography. For pressures up to 50 atm, use can be made of specially reinforced μl-size syringes[27] which are now commercially available. Injection is carried out through a standard septum which is protected from the mobile phase by a sliding valve. This valve is opened only during the injection period. For operation at higher pressures, use can be made of specially constructed sampling valves which can position the sample in the stream[17,27]. Another alternative is to place the sample in a chamber and then direct the high-pressure mobile phase

flow through this compartment by operation of a number of standard high-pressure valves[3].

(b) Column oven

Many commercial liquid chromatographs have some provisions for thermostat control of the column to ensure reproducible operations. Accurate temperature control is much more important in supercritical work because in the critical region there is a large dependence of the partition coefficient on the temperature. The range of the temperatures in general use would be from ambient to around 300°C. Liquid chromatography, on the other hand, is generally operated at ambient temperatures. In general, most liquid chromatographic column ovens will, therefore, have to be redesigned to be able to meet the requirements for high-temperature operation and for good control at these elevated temperatures.

(c) Preheater/conditioning coil

For a mobile phase with its critical temperature above ambient levels, it is necessary to preheat the eluent to above its critical temperature before it is allowed to enter the column proper. This can be carried out in a separate preheater and/or in a conditioning coil mounted in the column oven. Both components are probably desirable for fluids with a critical temperature above 80°C. A conditioning coil alone is sufficient for fluids like CO_2 with a T_c of only 31.3°C.

(d) Pumps

For mobile phases with a vapor pressure of less than 1 atm at ambient temperatures, *i.e.*, which are liquid at atmospheric pressures, the same pumps can be used as those described for high-pressure liquid chromatography. The stream is then heated to the desired temperature prior to introduction into the chromatographic system proper.

A main concern in liquid chromatography is to have a pump with solvent-programming capabilities; in supercritical fluid work an important consideration of the pump is that its output can be pressure-programmed. In liquid chromatography, the pumps are constructed to operate with adjustable flow while the output pressure is observed as a dependent variable. In SFC, it is more important to be able to control the pressure and obtain the flow as the dependent variable. A good pump for supercritical work is the modified mercury displacement pump[28]. The large, high-pressure syringe-type plunger pumps which are now available commercially are also well suited for this purpose although some modifications are necessary to adapt this pump to pressure-programming operations. Our most recent pump combines the pressure intensification principle with that of the modified mercury displacement pump. However, instead of mercury, steel pistons with spring-loaded Teflon seals are used to transfer the pressure from the high-pressure nitrogen source to the mobile fluid. This pump has been used successfully to pump liquid CO_2 up to 300 atm[27].

Laboratory piston pumps are also used in this technique, but a (multiple) damping system to decrease the size of the pulsations and a pressure regulator to

control the flow may be necessary. CASHAW[14] described a mobile phase metering system based on this principle.

Most of the pumps can only operate with compounds which are liquid at room temperature and atmospheric pressure. Modifications are necessary to make them useful for fluids which are in the gaseous form at ambient conditions. In this case, one can compress the gas with, *e.g.*, an air-driven diaphragm compressor and feed the mobile phase through a pressure regulator to the chromatographic system[3]. An alternative method to pump low-boiling compounds, *e.g.*, CO_2, is to use a large reservoir of the fluid held at a temperature above its critical temperature. It will be difficult, however, to carry out pressure-programming with this approach.

(e) Pressure-programmer

A pressure-programmer which has been used for systems powered by high-pressure gas has been described earlier[13]. A hand-loaded, high-pressure regulator, which has a small orifice and accurately controlled outlet pressures at very low flows, is used to load up the reference side of a higher-capacity dome-loaded pressure regulator whose output is used to control the inlet pressure to the chromatographic system. The initial outlet pressure can be set manually on the control regulator at any point in the desired operating range. A variable speed motor rotates the setting of this regulator. This increases the pressure in the reference side of the dome loader, proportionally increasing the output pressure to the pump and hence to the chromatographic system.

(f) Detectors

The detection step in SFC is currently the weakest link in the technique. The detectors in use have found prior application in GC or in liquid chromatography. Since these systems generally operate at ambient pressures, the supercritical column effluent has to be reduced to atmospheric pressure before detection can be carried out. Decompression usually takes place in a system of small-diameter capillaries and a pressure-reducing micrometering valve. The problems associated with this step have been touched upon earlier. If the effluent is gaseous at ambient conditions, high-molecular-weight solutes tend to form large clusters or fine droplets during the decompression stage, which makes detection by the standard GC detectors erratic and unpredictable. GIDDINGS *et al.* report, *e.g.*, an unsuccessful attempt to use the flame ionization detector when CO_2 was used as the supercritical mobile phase[20]. A possible solution to the problem is to subject the effluent to pyrolysis conditions at the outlet of the chromatographic column. This approach would hopefully reduce macromolecules to smaller and more volatile species which would form true solutions with the gaseous phase after decompression. Limited data by MYERS AND GIDDINGS suggest that this approach should be quite useful for many compounds[3].

With a low-boiling mobile phase which is liquid under ambient conditions, dissolved gases and part of the mobile phase may flash off, creating bubbles which would interfere with the detection process. Some solutes may even precipitate during this decompression stage. A gas removal section, prior to the detector, solves this problem but introduces additional band spread. However, once the effluent is obtained as a

homogeneous phase at atmospheric pressures, detection should be straightforward.

If the mobile phase has a relatively low vapor pressure at ambient conditions, problems are usually not observed when the pressure is reduced. However, as mentioned earlier, the use of these mobile phases is limited to solutes which are stable at the high temperatures necessary for the eluent to become supercritical. In this case, almost any detector which has been successfully used in high-resolution liquid chromatography should suffice. Most recently, a microadsorption detector has been reported as having been used successfully as the detector in an SFC system[1,14].

Most of the work in SFC, however, involves the use of an ultraviolet absorption detector. Even though its applicability is limited to solutes which absorb in the ultraviolet, this detector can be used in a surprisingly large number of separations. An important consideration is that SFC is especially applicable to the analysis of heavier molecules. These compounds have a high probability of possessing an ultraviolet absorbing chromophoric group in their molecular structures.

The absorptivity obviously varies from compound to compound. In the 200- to 300-nm range, it is as low as 10 for aliphatic olefinic systems and as high as 10^5 for polynuclear aromatic hydrocarbons. The mobile phases should be transparent in the wavelength region of interest. The UV absorption detector is very sensitive and also easy to use. Almost any UV recording spectrophotometer can be modified to perform as a detector, and quite a number of inexpensive units are now commercially available[30].

Detection is carried out in tiny flow-through cells. Detector cells with 10-mm path lengths and a cell volume as low as 10–20 μl are now quite common.

There is an alternative to decompressing to ambient pressures and encountering the problems described earlier. One can also construct a high-pressure detector cell which is mounted before the decompression section of the chromatographic system and analyze the effluent with the mobile phase still under high pressure. A UV detector cell, which has been used successfully up to 290 atm, has been reported[27]. This cell can, therefore, be used directly on the outlet of the chromatographic column prior to the decompression stage of the system[27].

An interesting design for a chromatographic detector has recently been patented by BROERMAN[31]. The outlet of the chromatographic column is directed towards a temperature-sensitive element which is mounted in a stream of flowing gas. By measuring the changes in cooling caused by the evaporation of the chromatographic effluent, an indication is obtained of the changes in composition of this stream. Although this detector has been developed for liquid chromatography, it should have equal applicability when the mobile phase is a fluid in the supercritical state.

5. APPLICATIONS

KLESPER *et al.* appear to be the first investigators to report a column chromatographic analysis with a supercritical fluid as the mobile phase[6]. In this paper, they described the separation of Ni etioporphyrin II from Ni mesoporphyrin IX dimethyl ester with dichlorodifluoromethane (T_c = 111.5°C, P_c = 39.9 atm) or monochlorodifluoromethane as the eluent. In subsequent communications, KARAYANNIS *et al.* described the behavior of a large number of porphyrins, etioporphyrin II metal chelates[32,33], metal acetylacetonates[34], and other metal chelates[25] when chromato-

graphed with dichlorodifluoromethane. Most of the work was carried out on a column packed with 10% Epon 1001 resin on Chromosorb W at temperatures between 115–145°C and pressures between 55–115 atm. The chromatograms shown were, however, not very encouraging because very large band spreads were observed with these compounds under these conditions of analysis.

Soon after the first paper had been published, several patent applications were filed in Austria for a separation process based on the enhanced solution of mixtures of organic compounds in a supercritical fluid[35]. By decreasing the pressure and/or by increasing the temperature of the system, a portion of the dissolved material is precipitated and separated out. It was, however, not until a few years later that these same applicants filed a patent application for a column chromatographic process with a supercritical fluid as the mobile phase[36]. With ethylene as the eluent and using stepwise pressure-programming, *n*-hexadecane, hexadecene, and octadecene were completely separated from each other on a column packed with alumina. 2-Ethylhexanol and benzyl alcohol were separated on a column of Sterchamol.

The most comprehensive study on this subject was published by SIE *et al.* in 1966 and 1967 on investigations which they had completed a few years earlier[5,7,11,16,17]. These papers are not only valuable because of the fundamental treatment of the subject but also because of the large number of analytical applications included in the text. With CO_2 as the carrier fluid, several mixtures of paraffinic and aromatic hydrocarbons have been chromatographed on a column packed with squalane on Sil-O-Cel. Most of the more interesting separations were, however, carried out with either pentane at 213°C or with isopropanol at 245°C as the mobile phase. With PEG 6000 or Alkathene on Sil-O-Cel as the stationary liquid phase, separations were reported of the phthalates in the C_{14}–C_{68} molecular-weight range both according to molecular weight and according to type[15]. Separations were also reported of several mixtures of aromatic hydrocarbons in the C_6–C_{20} range, polyphenyls in the C_6–C_{30}

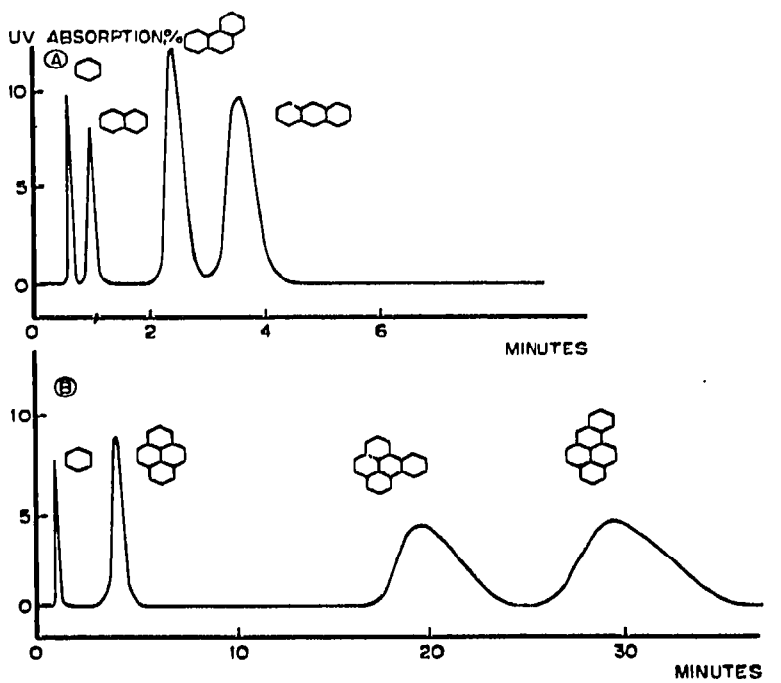


Fig. 5. Separation of isomeric condensed polynuclear aromatic hydrocarbons[6].

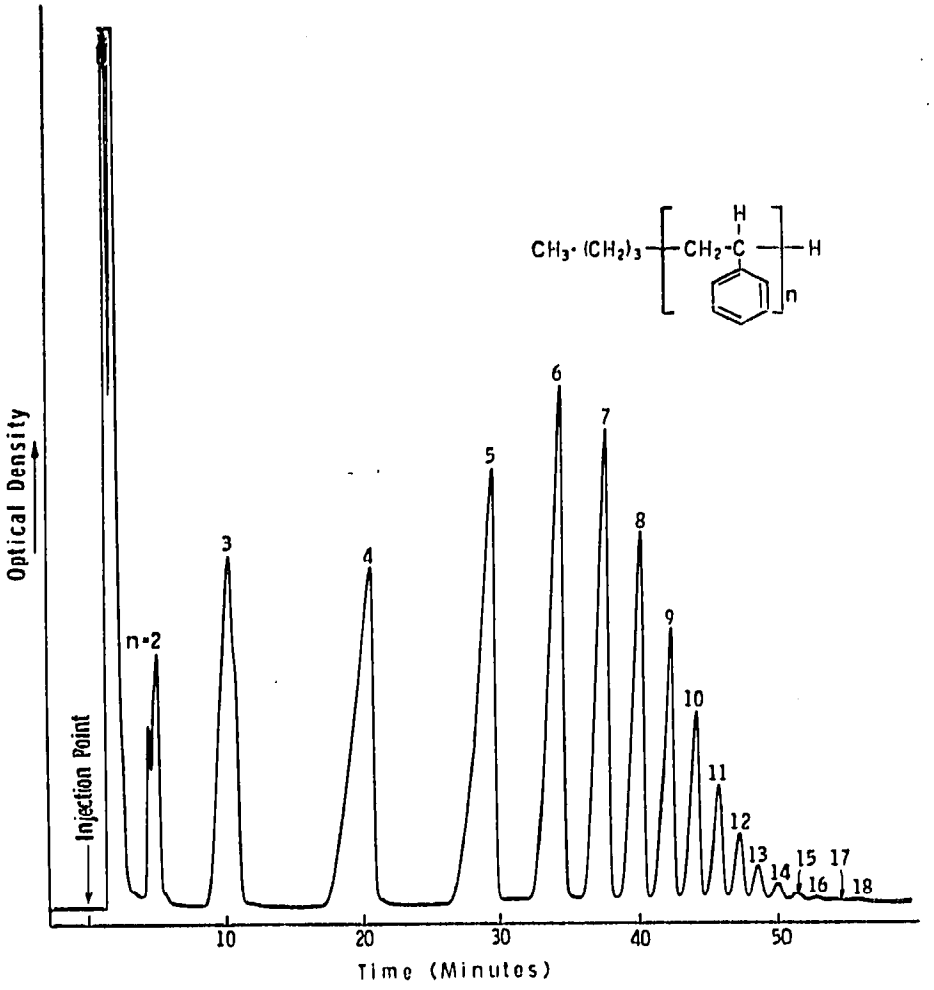


Fig. 6. Separation of the oligomers of a polystyrene mixture with a nominal mol. wt. of 600.

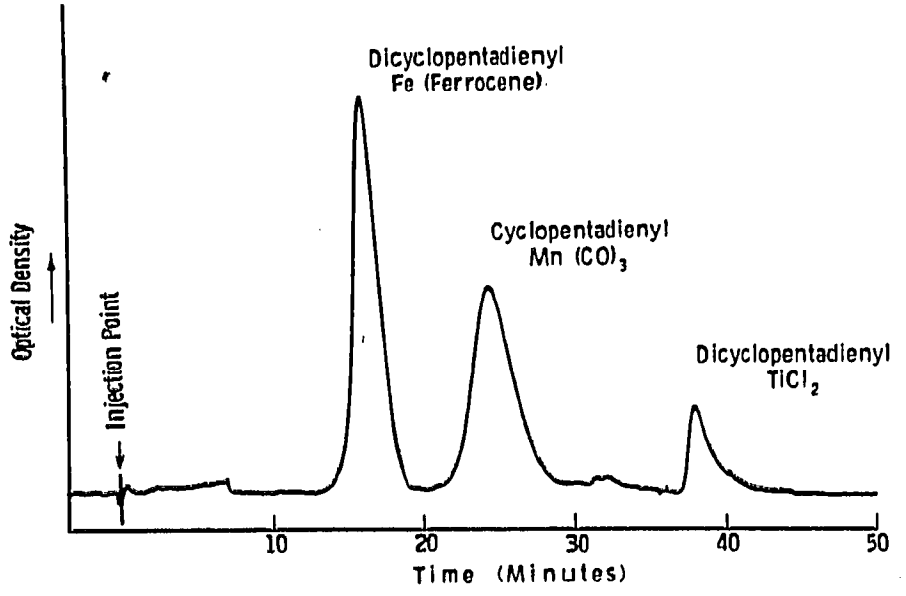
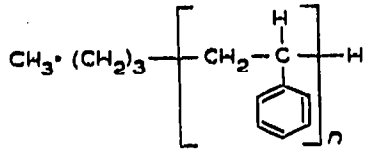


Fig. 7. Separation of ferrocene (dicyclopentadienyl Fe), cyclopentadienyl $Mn(CO)_3$ and dicyclopentadienyl $TiCl_2$ (ref. 27).

range, and of two isomeric C_{20} binaphthyls. Examples were also given of the separation of oxygenated compounds of near-equal molecular weight in the C_{22}–C_{26} range.

With alumina as the solid substrate, SF–solid chromatographic separations were reported of a number of condensed aromatic hydrocarbon mixtures. The most spectacular separations obtained were those between the isomeric condensed polynuclear aromatic hydrocarbons phenanthrene and anthracene (Fig. 5A) and between 1,2- and 3,4-benzpyrene[5] (Fig. 5B). These analyses were carried out in just a few minutes on a column showing only 200–250 theoretical plates. These separations are remarkable because on common isotropic phases, such as Apiezon L, silicones, etc., in the temperature range between 105–300°C, the relative retention in GC of the first-named isomer pair is close to unity. Very little or no separation is observed in packed columns. In capillary column GC, separations have only been reported as possible on very long columns and, interestingly enough, on a short capillary column[37]. The separation of 1,2- and 3,4-benzpyrene is also not easy to accomplish[38]. Even on long, packed columns only a partial separation of these two isomers is generally observed[5,37]. Fig. 5 shows the chromatograms which were obtained by SIE *et al.*

Another remarkable separation, which was reported a few years later, is shown in Fig. 6. The chromatogram is that obtained by a SF–liquid chromatographic separation of the oligomers of a polystyrene mixture with a nominal molecular weight[30] of 600. The product was obtained by anionic polymerization of styrene with butyl lithium as initiator and methanol as the terminating agent. The molecular structure can be described by



where n is the number of styrene molecules in the polymer. The separation was carried out with a pentane–methanol (20:1) mixture as the mobile phase on Porasil C to which *n*-octyl groups have been bonded as the stationary substrate. The temperature was maintained isothermally at 205°C. The pressure was maintained isobarically at 600 p.s.i.a. for 8 min and then programmed at 6 p.s.i./min to 900 p.s.i.a. This separation shows the power of pressure-programmed SFC; the oligomers shown range in molecular weight from 266 to 1930.

Other applications of pressure-programmed SFC were shown in a subsequent paper[13]. Separations were described of a mixture of polynuclear aromatic hydrocarbons, a mixture of polyphenyls in the 332 to 729 molecular-weight range, and commercially obtained "monodisperse" polystyrene mixtures with nominal molecular weights of 900 and of 2100. In the last-named example, the oligomer with $n = 32$ (mol. wt. = 3386) was eluted in about 70 min while a base line separation could still be observed between the oligomers with $n = 12$ and $n = 13$.

Lest it be implied that the analysis of higher-molecular-weight compounds by fluid chromatography can only be carried out at elevated temperatures, we have included a chromatogram of thermally labile ionic compounds obtained at a temperature slightly above ambient levels. Fig. 7 shows some metal organic compounds

which we have chromatographed at 40°C using CO_2 as the supercritical mobile phase[27]. The column was packed with Porasil F to which Carbowax was bonded. The pressure was maintained at 1100 p.s.i.a. for 22 min to effect the separation between ferrocene and cyclopentadienyl $Mn(CO)_3$ and subsequently pressure-programmed at 120 p.s.i./min to elute the dicyclopentadienyl $TiCl_2$.

6. CONCLUSIONS

Although SFC is only a few years old, it has already shown considerable promise as a rapid chromatographic tool for the analysis of medium- to heavy-molecular-weight compounds. In a number of cases, exceptional results have been observed which could not have been attained by other chromatographic techniques. The development of SFC has not been rapid, because of a variety of factors. For many applications, the detector section of the chromatographic system is a major problem which has not been solved adequately. The high pressures involved may have frightened some investigators away. Another important reason appears to be the explosive growth of high-resolution liquid chromatography, which has overshadowed SFC and decreased the efforts of scientists to work in this field.

SFC will obviously not displace either GC or liquid chromatography except in a number of selected fields and in some special applications. In one general area, however, SFC has a definite advantage over its sister techniques. *Quantitative* recovery of the separated solutes is relatively simple in SFC.

In preparative GC, the small amounts of solutes in large volumes of carrier gas make the quantitative recovery of these compounds extremely difficult. There must be at least one hundred papers describing different trapping designs and techniques, which attest to the magnitude of the problems encountered. In liquid chromatography, the problem lies mainly in the complete removal of the mobile phase. This problem is, of course, also present in SFC if high-boiling fluids are used as the mobile phase. In SFC, however, lower-molecular solvents are generally employed. With CO_2 as the mobile phase, for instance, quantitative recovery of the solutes is possible with a relatively simple high-pressure trapping arrangement[27].

Complete supercritical fluid chromatographs are currently not yet commercially available. Instrument manufacturers will probably make the first models available as options to their existing lines of liquid chromatographs. In the final analysis, one instrument should be capable of performing both liquid chromatography and SFC.

Even though some time is necessary before the instrumental problems are solved and the dynamics of the chromatographic separation are better understood, we are of the opinion that SFC has a definite place in the future as a major instrumental method of analysis.

7. ACKNOWLEDGEMENTS

It is a pleasure to acknowledge the courtesy of Marcel Dekker, Inc., publishers of *Separation Science*, for permission to publish Fig. 5, Interscience Publishers, Inc., publishers of *Polymer Letters*, for permission to publish Fig. 6 and the American Chemical Society, publishers of *Analytical Chemistry*, for permission to reprint Fig. 7.

The authors also gratefully acknowledge the helpful discussions and advice of Dr. R. L. LeTourneau.

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