Trends in Supercritical Fluid Chromatography: 1997

Larry T. Taylor
Department of Chemistry, Virginia Polytechnic Institute, Blacksburg, VA 24061-0212

Dr. Larry Taylor is currently the coauthor of 10 patents in the areas of analytical and polymer chemistry and consults for both industry and government. He currently serves as an Associate Editor for the Journal of Chromatographic Science *and is a member of the advisory board of the* Journal of High Resolution Chromatography. *He and his research group periodically teach a short course on supercritical fluid extraction and chromatography sponsored by the American Chemical Society (ACS). Dr. Taylor has recieved the NASA Public Service Medal, the A.F. Clifford Faculty Service Award, and the Virginia Blue Ridge Section of the ACS G.F. Wise Award for achievement in the chemical profession and community service.*

He has authored over 200 refereed technical publications as well as numerous technical reports for the Department of Energy, Electric Power Research Institute, NASA, National Institute of Health, Aluminum Company of America, Environmental Protection Agency, Naval Surface Warfare Center, and others. He has directed the research theses and dissertations of 60 master's and Ph.D. students. His research programs have covered coordination chemistry, small molecule activation, coal liquefaction, development of coupled chromatographic–spectrometric analytical techniques, analysis of propellants for polymeric and nonpolymeric components, metal ion modified polyimides for electrical, adhesive, and magnetic applications, metal-coated polymers, and analytical–synthetic applications of supercritical fluids in the areas of polymer chemistry, environmental science, and pharmaceutical chemistry.

Abstract

The future of supercritical fluid chromatography (SFC) will focus more on the separation of moderately polar analytes with packed columns, modified CO_2, and a host of detectors (e.g., universal, element-specific, and spectrometric). Consequently, SFC will become viewed more like high-performance liquid chromatography (HPLC) and will, in fact, replace HPLC in a number of applications because supercritical fluids have both better mass transport properties than liquids and are less harmful to the environment than commonly used HPLC liquids. The same packed column used for HPLC can, for example, generally be used interchangeably for many SFC applications. The relatively poor solvating power of CO_2, however, has dictated in many cases the use of modifiers in the mobile phase. These additives may enhance the solvating power of the supercritical fluid and deactivate or modify the stationary phase. Packed columns that have much higher decompressed flow rates than open tubular columns will place new demands on the employment of postrestrictor detectors in SFC.

Introduction

The properties of gas-like diffusivity, gas-like viscosity, and liquid-like density combined with pressure-dependent solvating power have provided the impetus for applying supercritical fluid technology to analytical separation problems. Supercritical fluid chromatography (SFC) is an analysis technique that uses supercritical fluids as the mobile phase. Liquid chromatography-like separations that exhibit more gas chromatography-like figures of merit such as high speed, high resolution, and multiple detection options are characteristic of SFC with packed columns. On the other hand, open-tubular column SFC is an extension of gas chromatography (GC) to larger, less volatile, and less thermally stable

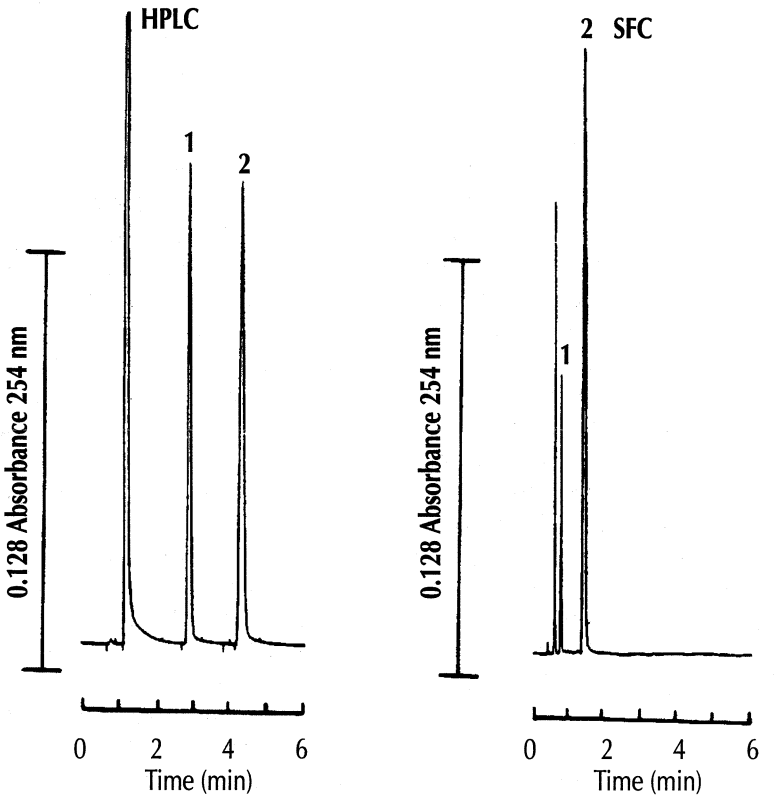

Figure 1. Chromatograms of the separation of biphenyl (peak 1) and pyrene (peak 2) at the optimum average linear velocity for HPLC and SFC. An ODS reversed-phase column (10 cm × 4.6 mm) and a 5-µm particle diameter were used. For HPLC, the solvent was acetonitrile and water (70:30) at 1.0 cm^3/min; linear velocity, 0.13 cm/s; and column pressure drop, 62 bars. For SFC, the carbon dioxide flow rate was 2.5 cm^3/min; linear velocity, 0.40 cm/s; column pressure drop, 14 bars; average column pressure, 165 bars.
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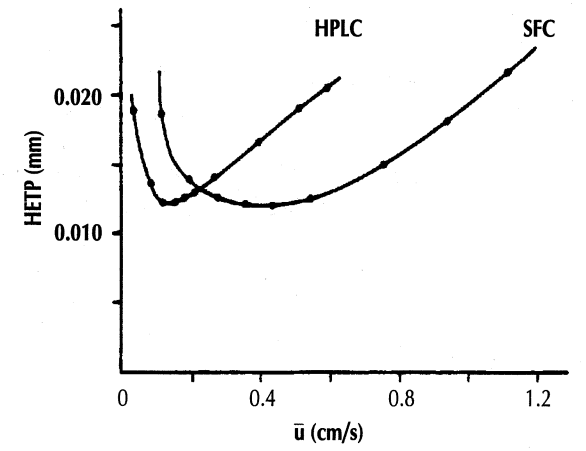

Figure 2. Van Deemter plots of chromatographic data for HPLC and SFC elution of pyrene; HETP is height equivalent to a theoretical plate. Conditions: 10 cm × 4.6-mm i.d. packed column, C_{18} bonded silica particles (Hypersil), 5 µm. HPLC: k' = 2.85, 30% H_2O in CH_3CN, 40°C. SFC: CO_2 at 0.8 g/mL, k' = 2.30.
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molecules. The approach to methods development varies greatly depending on the column type. Currently, it appears that the trend of SFC is more in the direction of both packed analytical scale and packed capillary columns as an extension of and possible replacement for normal-phase liquid chromatography (LC). This review will describe in a cursory manner some of the current trends regarding SFC.

Berger has stated that SFC may be usable with 30% of all molecules and that 20% of the total of LC instruments in laboratories will be SFC instruments (1). SFC is considered to possess inferior figures of merit compared to GC, but the technique of SFC is more widely applicable. On the other hand, SFC possesses superior figures of merit compared to LC, but SFC is less applicable than LC. It should be noted, however, that all the control parameters available in both GC and LC are available and useful in SFC (e.g., mobile phase composition and identity, temperature, pressure, flow, and stationary phase identity). Methods development should, therefore, be more straightforward in SFC than HPLC because it is more versatile in many situations.

For example, Figure 1 shows a comparison of the HPLC and SFC traces of biphenyl and pyrene at the optimum average linear velocity for each solute (2). An octadecylsilica (ODS) reversed-phase column (10 cm × 4.6 mm, 5-µm particle diameter) was used. In both cases, the SFC separation was completed in less than 2 min, whereas the HPLC separation required over 4 min. Experimentally-derived van Deemter plots from the HPLC and SFC elution of pyrene are shown in Figure 2. For the same packing material, the minimum height equivalent to a theoretical plate (HETP) was the same, regardless of whether a liquid mobile phase or supercritical fluid mobile phase was employed. The optimum SF linear velocity, on the other hand, was more than double the HPLC optimum linear velocity. Such time-saving relative to GC, on the contrary, does not exist with SFC because gases afford much higher optimum linear velocities than SFs. From a speed and efficiency standpoint, GC should be the first method of choice, SFC should be the second choice, and when neither of these techniques are applicable, HPLC should be selected based on HETP considerations.

In terms of economic and environmental issues (e.g., solvent price and disposal), SFC may again be preferred over HPLC. This advantage in analysis is particularly striking when solvent usage and sample throughput for SFC and HPLC of felodipine are compared (see Tables I and II) (3). When the SFC–ultraviolet (UV) system was used for analysis, sample throughput was increased by 60% over an analogous HPLC separation. Although more total mobile phase (by volume) was used for SFC than HPLC, the ability to run six additional samples by SFC per hour resulted in only 6% of the SFC mobile phase that could be considered as disposable solvent waste.

The remaining 94% was carbon dioxide gas, which was vented to a hood. The disposal cost incurred for 100% organic solvent (nonchlorinated) versus a mixture of water and organic solvent also illustrates another advantage of the SFC assay. The most common procedure for solvent waste disposal is combustion in large scale manufacturing furnaces. Such furnaces typically combust 45,000 gallons of solvent waste per hour. Because water–organic solvent mixtures generated from HPLC analysis produce less heat (less than 3000 Btu/lb) upon combustion, the resulting cost of disposal to the waste source is higher. Conversely, 100% organic solvent disposal (generated by SFC) has a higher fuel value (9500 Btu/lb); therefore, its cost of disposal is less.

Column Restriction

One unique feature of SFC relative to HPLC is the incorporation of a restrictor at the end of the column in SFC to maintain supercritical conditions inside the column. These restrictors may have fixed inner diameters or be electronically controlled variable restrictors. The effect of increased linear velocity on column efficiency can be very significant when passive fixed-diameter restrictors located at the column outlet are used. Under these conditions, higher operating density is achieved only at the expense of a greater pumping rate. The pump delivers whatever flow is required to achieve the column head pressure setpoint. In other words, density and flow rate are coupled when fixed restriction is employed. This situation has been described as operation in the "upstream mode" (1). Upstream control is most often used in situations that require very low flow rates. A worst-case scenario in the upstream mode develops during an SFC run with density programming. The optimum linear velocity decreases as the density increases because conditions become more liquid-like, whereas the operating linear velocity increases as density increases because the pump is working harder. Under pressure programming conditions, if one starts out at the optimum mobile phase velocity, one could easily end up at several times the optimum velocity by the end of the run. For example, with a 7-m × 50-µm-i.d. column at 40°C, the linear velocity goes from 1.3 cm/s at 0.47 g/mL to 10.2 cm/s at 0.96 g/mL using a fixed restrictor. If the column were operated at 100°C, the increase in linear velocity would be more than twice the optimum linear velocity (4).

High-pressure electronically controlled micrometering valves are becoming quite popular in packed-column SFC. These backpressure regulators, which are termed "variable restrictors," allow flow rates to be adjusted to constant levels at different densities (pressures). Because the pressure is controlled after the column, the mode of operation is termed the "downstream mode." In contrast to analytical-scale columns, capillaries and micropacked columns require low flow rates and have such low dead volumes that it is presently impossible to perform downstream control with existing equipment. The reader is referred to reference 4 for the designs of several variable restrictors. Currently, prerestrictor detectors (e.g., UV and Fourier transform infrared [FTIR]) and analytical-scale packed columns are applicable to variable restriction. Much of the future acceptance of SFC by separation scientists rests upon the universal incorporation by workers in the field of variable restrictors in which mass flow and pressure can be decoupled. Without a variable restrictor, it becomes extremely difficult from one separation to the next to deconvolute the individual effects of changing (*a*) the holdup time (t_m), (*b*) the fluid linear velocity, (*c*) the retention factor, and (*d*) the column efficiency.

Table I. Packed-Column SFC Versus HPLC*

	SFC	HPLC
Total retention time	4.34 (0.3%)	9.23 (0.5%)
Holdup time (min)	1.26	1.52
Retention factor	2.44	5.07
Peak width at half-height (min)	0.089	0.25
Plate number	13115	8519
Plate height (mm/plate)	0.017	0.029
RSD (peak area)	1.1%	1.2%

SFC analysis conditions: 25cm × 4.6-mm i.d. Hypersil Si column; temperature, 45°C; pressure, 300 bar; flow rate, 2 mL/min; injection volume, 5 µL; felodipine concentration, 1 mg/mL.

* All peak parameters were calculated based on 5 replicate injections of a felodipine standard. Values are given in parentheses.
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Table II. Solvent Usage Comparison for Analysis of Felodipine by Packed-Column (4.6-mm i.d.) SFC and HPLC

	Packed-column SFC–UV	Packed-column HPLC–UV
Mobile phase	6% (v/v) methanol-modified CO_2	Acetonitrile–methanol/50mM phosphate buffer (pH 3) (40:20:40, v/v/v)
Samples analyed per hour	10 (6-min run time)	4 (15-min run time)
Mobile phase used (mL) per sample analyzed	12.0	25.0
Disposable waste (mL) per sample analyzed	1.0	25.0
Mobile phase disposal cost per 55 gal	$48*	$175*

* Disposal costs obtained from Solid Waste Management, Merck Research Laboratories, West Point, PA.
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Mobile Phases

In lieu of increasing the solvent power of the SF by increasing the density, the solvent power of SF phases can be varied by the addition of polar compounds (i.e., modifier) to the primary fluid. Here

retention factors become not only a function of modifier properties but also of modifier concentration. The use of binary phase (two-pump) systems offers great analytical flexibility because the modifier identity and concentration can be easily changed. With this mobile phase pumping option, both isocratic and gradient delivery can be employed. Methanol is by far the most commonly used modifier in SFC. Acetonitrile, which has a higher polarity index than that of methanol and is less soluble in CO_2 than methanol, is less frequently used. Water is even less soluble in CO_2 than acetonitrile; therefore, when water is used as a modifier in CO_2, the mobile phase is usually saturated with water.

The effects of modifiers in SFC are as follows: (*a*) they cover active (silanol) sites, (*b*) they swell or modify the stationary phase, (*c*) they increase the density of the mobile phase, and (*d*) they can be used to increase the solvent strength of the mobile phase (5). This multifold mechanism of action for modifiers in SFC, however, is somewhat ambiguous and results in competing mechanisms (i.e., stationary phase effects versus mobile phase effects). It has been reported that, in contrast to packed columns, open tubular (OT) columns do not show the drastic changes in retention factors or peak shape upon addition of small amounts (less than 2%) of modifier (4). These less drastic differences were attributed to differences in the degree of deactivation of the packed column stationary phase as compared with the OT column stationary phase. An OT column has a smaller number of active sites present; therefore, less active sites are present for the modifier to deactivate. However, Berger et al. contend that these modifiers produce about the same results in OT and packed columns. They reported that the retention of polyaromatic hydrocarbons (PAHs) decreased 15–32% on a variety of packed columns and 26–28% on OT columns when approximately 2% of 2-propanol or methanol was added to the mobile phase (5).

The modifier may be further altered by introducing low concentrations of a very polar compound. Thus, when the secondary modifier is added to the mobile phase via the primary modifier, it is sometimes referred to as an additive (6). Acetic, citric, chloroacetic, dichloroacetic, trichloroacetic, and trifluoroacetic acids have been used as acidic additives, whereas tetrabutylammonium hydroxide (TBAOH) and isopropylamine (5) have been frequently employed as basic additives. One role suggested for the additive in these separations is to ensure analyte neutrality (1).

For example, Berger et al. (7) have separated mono-, di-, and trihydroxybenzoic acids on cyanopropyl, diol, and sulfonic acid-derived silica columns. When pure carbon dioxide was used, none of the acids eluted from the columns. When methanol was added to the corresponding mobile phase, some of the candidate acids eluted but with very poor peak shapes. However, the addition of citric acid to the mobile phase allowed for the separation of 10 mono-, di-, and trihydroxybenzoic acids in approximately 1.5 min with much improved peak shape. The authors concluded that the additives' most predominant mode of action is to improve the solubility of the solute in the mobile phase and to suppress the ionization of very polar solutes. They also concluded that very polar additives interact so strongly with the active sites on a column that they serve to further deactivate the column.

For SFC systems, the mobile phase is simply a laboratory-sized cylinder equipped with a syphon tube from any number of manufacturers. Carbon dioxide, by virtue of its moderate critical parameters, high purity (SFC grade), and low cost, is the most commonly used SF today. The CO_2 is removed from the supply cylinder as a liquid and is pumped as a liquid. It has been common practice to add a helium headpressure to the supply cylinder so that the cylinder pressure is made greater than the vapor pressure of the fluid. The helium is intended to aid in pushing the remaining CO_2 liquid at the bottom of the tank up and out. Helium, however, dissolves in CO_2, thereby lowering its solvating power. Furthermore, the composition of the liquid CO_2–helium mixture changes as liquid is withdrawn from the cylinder because of the great difference in volatility between CO_2 and helium (8,9). Future SFC experiments should not use helium-padded fluids because padding is not necessary with properly designed pumping systems. Two major types of pumps are found in SFC instruments: syringe and piston. Syringe pumps have fixed volumes; therefore, dual syringe pump arrangements are employed so that, as one is being emptied, the other one is being filled. Piston pumps are only limited by the liquid volume of the gas–liquid supply cylinder. However, it is necessary to cool the piston heads in some manner so that only the noncompressible liquid phase is pumped, thereby avoiding cavitation at the pump head.

Modifiers can be introduced into chromatographic systems in primarily two ways. First, premixed tanks of modified fluid can be purchased with a variety of fluids and modifier percentages and are usually sold by weight percentage of modifier in pure fluid. Poor mixing and fluctuations in the modifier content for premixed CO_2 gas cylinders have long been suspected as a source of erratic chromatographic behavior in SFC (8,9). Although premixed cylinders allow some exploratory work to be accomplished with a single pump, the results often cannot be reproduced. Premixed binary fluids should therefore be avoided in SFC (9).

A second way to add modifier to a chromatographic system is to use a two-pump system in which one pump delivers the pure fluid and the other pump delivers the liquid modifier. The two fluid streams are mixed in a volume–volume ratio to form the mobile phase. This method of preparing the mobile phase provides the greatest flexibility in that the mobile phase is mixed in-line. It is also an accurate way to add modifier because the flow rate of each pump is known. Usually a reciprocating piston pump is used to deliver the pure fluid, and a syringe pump is used to deliver the modifier. One of the main concerns in mixing a liquid modifier with a supercritical fluid is that the compressibilites of the two fluids are different. The compressibilities of the fluids are of a special concern if a pressure gradient is to be used. For example, if a pressure gradient at a constant composition is required, then as the pressure increases, the relative speed of the syringe pump delivering CO_2 decreases compared with the speed of the syringe pump delivering the less compressible organic modifier (10). Because the modifier concentration in SFC is generally small, the main fluid pump and the modifier pump will also operate at different rates. Vendors have, for the most part, solved this problem.

The solubility of the modifier in the supercritical fluid is also important. If the solubility of the modifier is exceeded, a two-phase

(liquid vapor) system will result, and the effect of a two-phase system on the chromatography may be detrimental. A UV detector often gives an indication of whether a two-phase system is present or not; a very noisy signal results when two phases are present. High solubility is not required for SFC, but chromatography obviously becomes impossible without some finite solubility. Mixtures of two miscible fluids such as CO_2 and methanol usually do not become instantaneously homogeneous after the mixing point. Therefore, a packed bed of stainless steel balls downstream of the mixing point usually suffices as a mixing column.

Injection

The most common injectors for SFC are high-pressure valve injectors similar to those used in HPLC (11). With these valves, the sample is loaded at ambient pressure in a sample loop of defined size (100–500 nL). Direct full loop injections are the normal means of sample introduction in SFC with packed columns. After the sample is loaded into the sample loop, the valve is manually or pneumatically actuated. This operation places the sample loop in-line with the high-pressure mobile phase flow. The loop is purged with liquid CO_2, which becomes supercritical upon entering the heated zone and column. Injections should be made at low pressure and ambient temperature because pressure programming will normally ensue. Complex phase behavior phenomena may exist during the injection process at the injector and at the head of the column because the sample injection solvent is almost always an organic solvent. Peak splitting or peak shoulders can be avoided by ensuring complete mixing of the injection solvent and fluid.

The most common method currently used for injection with OT columns is split injection. Accuracy and precision are usually much better with time-split injection than with dynamic flow split injection, especially with internal standards. Chester and Innis (11) have reported that the key to successful quantitative analysis is the complete avoidance of splitting the sample. If everything is done correctly, precision should be limited only by the correct filling of the injection valve loop and should equal the precision of HPLC. These investigators have developed an injection approach relying on the formation of a liquid film of the injection solvent on a retention gap and the subsequent refocusing of the solutes from the flooded zone onto the head of the column. The entire sequence is illustrated in Figure 3. With this approach, volumes up to 0.5 mL were injected by Chester and Innis with little or no sacrifice of chromatographic performance while achieving relative standard deviations (RSDs) of less than 0.3% for the injected volume. RSDs for solute area were only slightly worse, depending on the peak shape.

Packed columns pose much less of a sample injection problem because of their greater capacity, and direct injection methods have proven highly effective and reproducible for packed column use. In fact, for packed columns of 4.6 × 1.0-mm i.d., direct injection (e.g., 5–10 µL) is as routine and reliable as is found for HPLC injection. The only difference between the two techniques is that the injection solvent can never be the mobile phase unless one is performing supercritical fluid extraction directly coupled to SFC (SFE–SFC). Large sample volume injections, however, are to be avoided unless a solvent

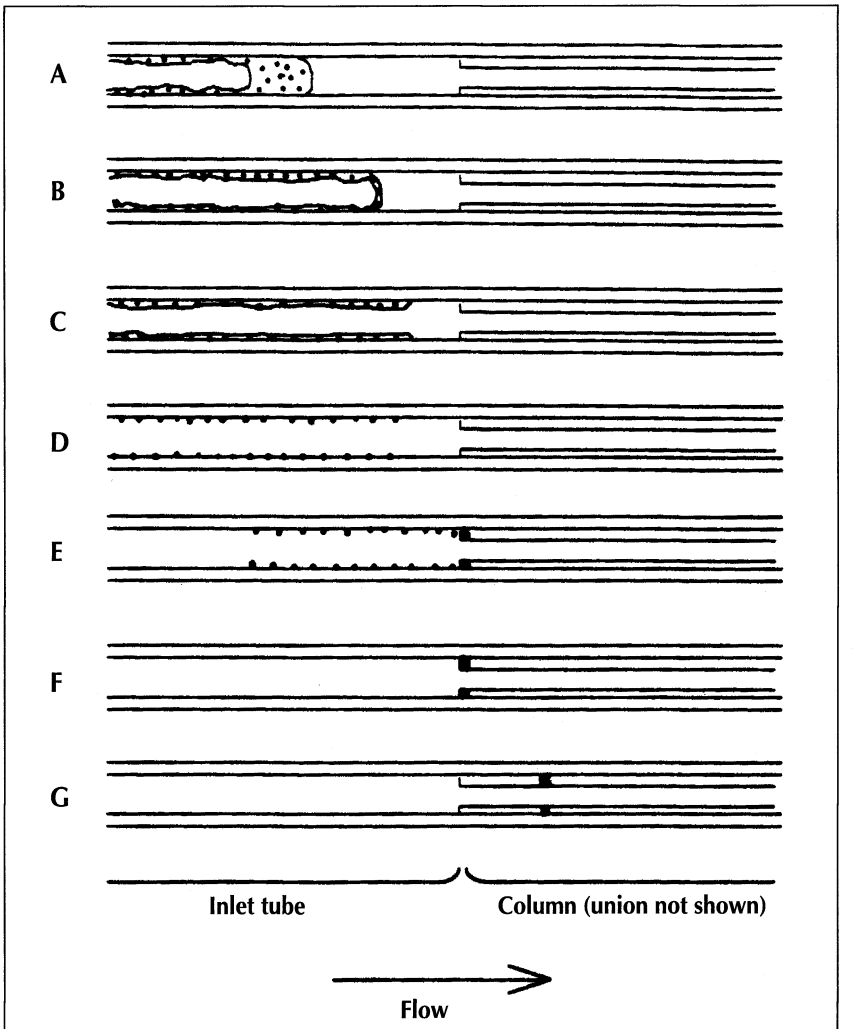

Figure 3. Representation of sample spreading due to inlet flooding and the refocusing effect possible with the use of an uncoated inlet tube. (A) Solute is carried over a length of inlet tubing (or column) by the liquid injection solvent. (B,C) Sample spreading continues until injection solvent is depleted (or sufficiently diluted). (D) Solvent-free solute is left spread over what was the flooded zone. (E,F) If flooding occurs on an uncoated inlet tube, solute migration will begin at a mobile phase strength too low for significant migration on the column stationary phase. Solute reaching the stationary phase is refocused into a narrow band. (G) Solute migration along the column begins as the mobile phase strength is raised further.
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elimination protocol is introduced prior to the separation. Excessive use of injection solvent may cause band-broadening and peak-splitting, and the properties of the stationary phase may change.

Stationary Phases

OT coated capillaries (50–100-mm i.d.), packed capillaries (100–500-mm i.d.), and packed columns (1–4.6-mm i.d.) have all been used for SFC. Conventional GC OT columns are not used in SFC because their column inner diameters are too large, and their stationary phases, which have not been extensively crosslinked, migrate on the column under supercritical conditions. On the other hand, HPLC columns are routinely and interchangeably used for SFC.

Many silicone stationary phases developed for either GC or HPLC have been adopted for use in SFC. These include phases exhibiting all types of solute–stationary phase interactions and selectivities such as adsorption, dispersion, dipole-induced dipole, dipole–dipole, size, and shape. The most widely used polar phase in OT columns is cyanopropyl–polysiloxane (12).

In packed columns, the stationary phase is normally near monomolecular thickness and is chemically bonded to the support. Particle sizes vary from 3 to 10 µm in diameter, and pore sizes range from 100 to 300 Å, which corresponds to a surface area of 100–300 m^2/g. Packed column stationary phases are silica-based and may either be of the ordinary HPLC type, in which the phase is bonded to the support, or the polymer-coated type, in which the phase is both bonded to the support and subsequently polymerized. The surface activity in the LC case is a serious limitation when mobile phases of low polarity (such as CO_2) are used. The silica surface activity appears to be directly related to (*a*) the number of silanol sites remaining after bonded phase application, (*b*) the accessibility of these silanol sites, and (*c*) the degree to which these residual silanols are covered with physically adsorbed mobile phase. With supercritical CO_2, silanol sites are essentially uncovered. Many workers have shown that conventional endcapping is not effective because all silanol sites are not reacted, and at temperatures above approximately 120°C, the endcapping reagent is not stable.

A more satisfactory solution to this dilemma, especially developed for SFC, involves the use of hydrosiloxane polymers that are coated and chemically bonded to a porous silica particle (13). A surface coating technique similar to one previously developed for capillary GC was employed. The separation of a mixture (4 µg/µL each) of *n*-pentadecane, phenyl acetate, acetophenone, 2,6-dimethylaniline, and phenol on both a regular cyanopropyl and a crosslinked cyanopropyl-coated packed column is shown in Figure 4. Peak shapes were much improved on the crosslinked phase, especially for 2,6-dimethylaniline, the most basic component. All polar solutes exhibited some tailing on the regular cyanopropyl column. The difference in holdup time between the two separations can be attributed to the fact that the silica base, pore size, and pore size distribution are somewhat different for the two columns.

Packed column stationary phases are finding improved utility for chiral separations, which are important in the pharmaceutical industry (14). The two most popular chiral stationary phases are the Pirckle phases, in which π-interaction and hydrogen bonding prevail, and the cyclodextrin phases, in which an inclusion complex interaction between the solute and the stationary phase is thought to dominate. Cellulose derivatives coated onto silica gel are also used as stationary phases. With packed columns, most of the separations are performed under subcritical conditions because enantiomeric selectivity is usually enhanced at low temperatures. Figure 5 shows the chiral separation of two phosphine oxides (e.g., 2-naphthyl and *o*-anilyl) employing a liquid mobile phase and a subcritical mobile phase (15) with a cyclodextrin stationary phase.

By combining the advantageous features of both packed and

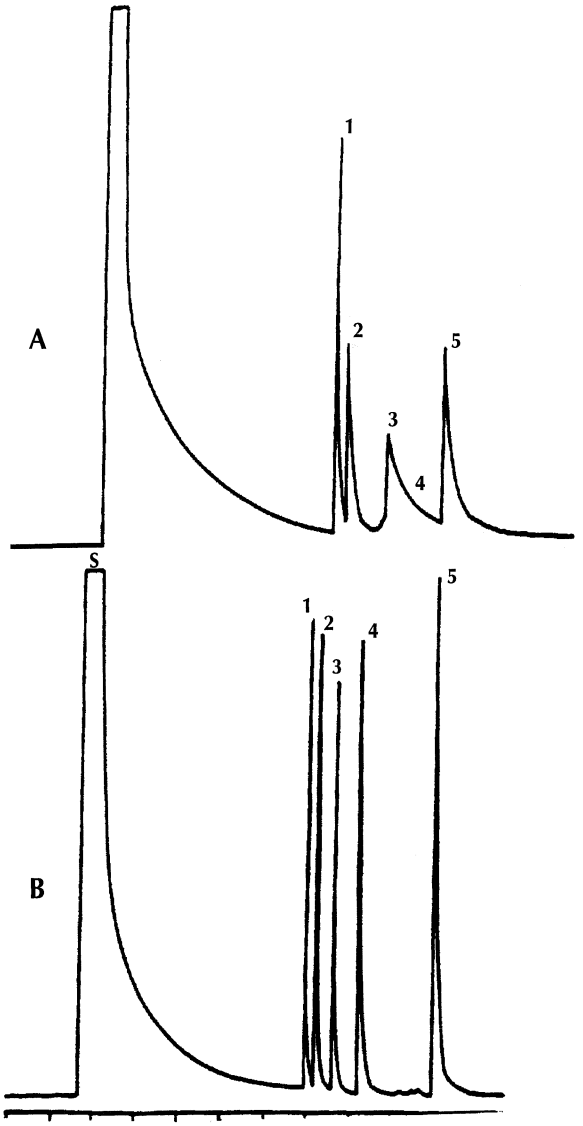

Figure 4. Separation of polarity test mixture on (A) conventional cyanopropyl and (B) polymer-coated cyanopropyl columns with 100% CO_2 and flame ionization detection at 60°C. Peaks: (1) *n*-pentadecane, (2) phenyl acetate, (3) acetophenone, (4) 2,6-dimethylaniline, and (5) phenol.
(Reprinted with permission from reference 13.)

OT columns, packed capillaries open new possibilities in high-performance SFC separations. Because of the inherent low flow rates, packed capillary columns have significant advantages over conventional packed columns for use in hyphenated systems like SFC with mass spectrometric detection (SFC–MS). Packed capillary column SFC will also provide remarkable economy in SFC operation, requiring mobile phase quantities a few orders of magnitude lower as compared with conventional packed-column SFC. Many excellent papers on the potential of packed capillary SFC have been published in recent years (16–19).

Detection

A major advantage of SFC over HPLC is that SFC is truly multidetector-compatible. Both HPLC and GC detectors have been successfully interfaced in SFC. Detection may occur before the restrictor by using a closed cell design in which the fluid is maintained under pressure, or alternately, detection may occur after decompression to a gas (i.e., postrestriction).

The most useful SFC detector is flame ionization (20) when CO_2 is used as the mobile phase. The flame-ionization detector (FID) responds to most organic molecules (detection limit for carbon: 1–10 pg/s) and exhibits a linear dynamic range of 10^6. Gas flow rate and restrictor design and placement are critical. The flame structure is larger for carbon dioxide than helium. Therefore, the collector position and voltage must be optimized. Because Joule-Thomson cooling accompanies CO_2 decompression (e.g., expansion), the temperature of the FID should also be higher than those used in GC. In addition to its sensitivity, FID is amenable to pressure programming, is easy to operate, does not respond to CO_2, and is applicable to both OT and packed columns.

Electron-capture detection (ECD) is highly sensitive to compounds with high electron affinities (e.g., halogenated compounds). In SFC, the decompressed mobile phase flow rate must be low; consequently, OT columns were demonstrated first where the flow is 1–10 mL/min. A makeup gas of 10% CH_4 and 90% Ar is necessary in order to enhance the diffusion of thermally excited electrons in CO_2 and to achieve a stable baseline. Density programming is possible for this concentration-sensitive detector. Both associative and dissociative electron-capture mechanisms are in operation; therefore, the detector temperature is critical (e.g., 200–400°C). This detector has now been demonstrated with packed columns and modified fluids (21).

Additional postrestrictor detectors have demonstrated feasibility with SFC. Chemiluminescent detectors, which are specific for nitrogen and sulfur, are especially attractive. These detectors were initially developed for GC, but they are beginning to find application in SFC. They operate first by combusting the nitrogen- (or sulfur-) containing sample eluting from the column. These combustion products then react with ozone to produce an excited-state species, NO_2 (or SO_2), which results in chemiluminescence. An equimolar response for all nitrogen (or sulfur) compounds has been observed to date. Shi et al. (22) have described a packed-column SFC system with chemiluminescence nitrogen detection (SFC–CLND). They have shown that the response of the detector decreased as the amount of methanol modifier increased from 0 to 15% methanol-modified CO_2. The CLND signal was also found to decrease by increasing the decompressed CO_2 flow rate. An optimum flow of 150 and 90 mL/min was reported at modifier concentrations of 1–5% and 10–15%, respectively. A postcolumn split was used with 4.6-mm-i.d. columns to allow a slower flow rate to be delivered to the CLND.

Flame photometric detection is also sulfur-specific,

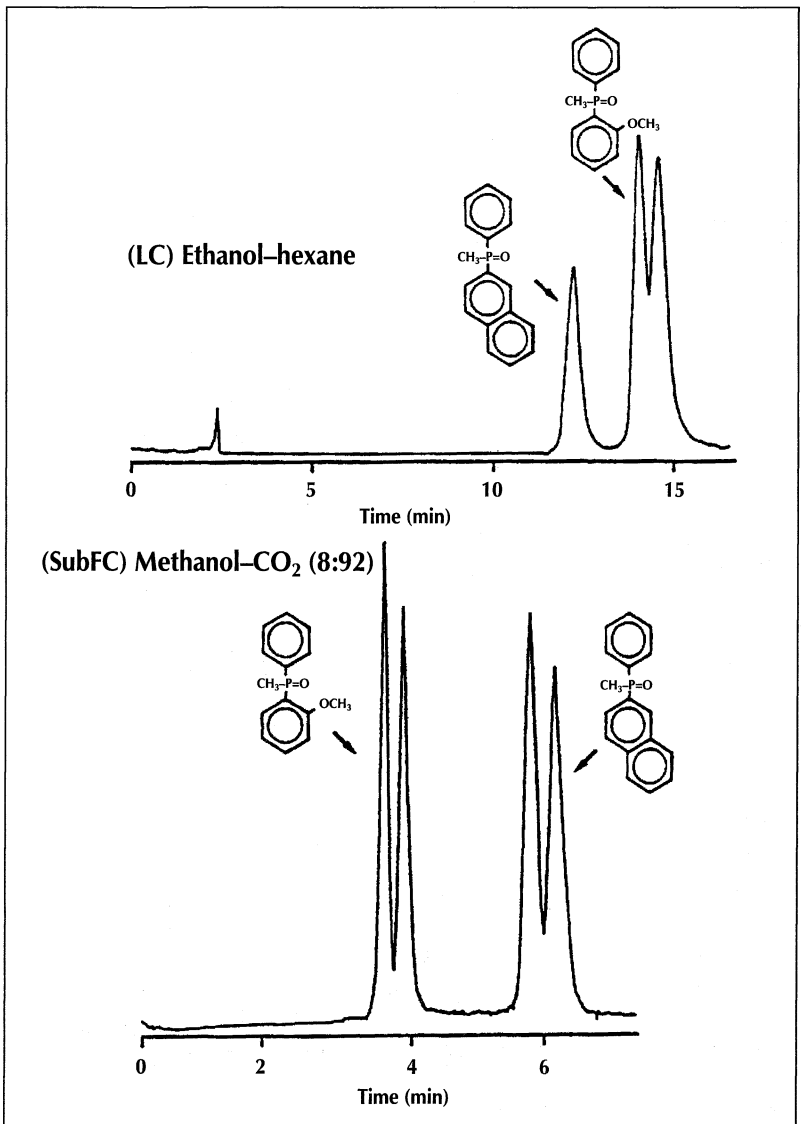

Figure 5. Chiral separation of phosphine oxides on cyclodextrin stationary phase employing liquid chromatography and subcritical fluid chromatography. *(Reprinted with permission from reference 14.)*

but response varies with CO_2 density and depends on the sulfur content in the compound, in contrast to sulfur chemiluminescent detection. Photoionization, ion mobility, and thermionic detection have also been shown to be feasible for SFC.

The second most popular detector for SFC is UV detection. With packed columns, this prerestrictor detection is simple, whereas with OT columns, the interface dead volume is a serious issue. Regardless of the column, certain trade-offs have to be made in flow cell design to achieve small flow cell volumes and relatively long flow cell pathlengths. These compromises are not severe for packed columns, but for OT columns, several strategies have been attempted, such as on-column detection and pseudo on-column detection (23). Neither of these strategies have proven very satisfactory, and the failure of OT columns with UV is an area in which improvements are needed. With prerestrictor detectors, analysis is usually performed in the liquid state rather than the supercritical state because solute concentration is higher, and the collimation of light is more effective. Related to the UV detector but much more sensitive and selective is the fluorescence detector, which has been shown to be feasible with SFC.

Several evaporative light-scattering detector (ELSD) interfaces have been described for use with packed-column SFC (24–28). The responses of ELSD interfaces were affected by the restrictor type, drift tube temperature, nitrogen makeup gas flow rate, and modifier concentration. The signal was found to decrease as the detector temperature was increased. Sensitive detection (less than 10 ng) was found to be possible. Packed-column SFC–ELSD seems to be a promising technique for assaying foodstuffs, polymers, drug substances, and products of pharmaceuticals. It will be especially useful when organic modifiers and additives are required that prohibit the use of universal FID detection. The usefulness of orthogonal separation methods (packed-column SFC–ELSD versus HPLC–UV–vis) has been demonstrated.

MS detection is also practiced with SFC. Traditionally, MS has been most studied with OT columns; however, packed column SFC–MS is gaining in popularity (29). A variety of ionization sources have been used, but chemical ionization is the most common. Progress is rapidly being made with common HPLC–MS interfaces such as thermospray (30), particle beam (31), and atmospheric pressure ionization (32) for use with packed columns. SFC presents an attractive method for samples not amenable to either GC or LC, but it is also useful for routine assay. No longer should SFC be a last resort technique; it should be exercised to its full advantage, especially when coupled to MS. With continual advances in commercially available instrumentation, sample assay by SFC–MS should become routine and rugged.

Conclusion

Supercritical fluids possess many of the attributes necessary for achieving good chromatographic separation. More importantly, by changing the density of the mobile phase with a change in temperature and/or pressure, the observed chromatographic characteristics in an SFC separation can be changed. Thus a single supercritical mobile phase can be used to afford a wide variety of separations without the often time-consuming column equilibration that is necessary in HPLC when changing mobile phase composition. Carbon dioxide is by far the most common mobile phase used in SFC. Polar compounds are widespread and are often nonvolatile and thermally labile. These properties make analysis by GC impossible without derivatization and can make method development for LC complex. SFC does not require solutes to be volatile because separations are carried out at low temperatures, and method development is straightforward. However, supercritical carbon dioxide is nonpolar and therefore will not solvate many compounds of interest. The poor solvating powers of supercritical CO_2 for polar compounds necessitates the addition of a mobile phase modifier such as methanol. The future of SFC at this time appears to be bright. The focus of applications and research will be on traditional packed columns, modified CO_2 mobile phases, and the application of numerous detectors.

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