

# Supercritical Fluid Chromatography and Extraction

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Interest and application of supercritical fluid chromatography (SFC) continue to grow. In the past there was a blur rather than a sharp dividing line between open-tubular SFC and gas chromatography (GC). The reports of SFC in these reviews were included in the GC section during the 1980s. In the last two years, significant growth has occurred in packed-column SFC as the introduction of new commercial instruments, coupled with significant new reports in the literature, has spurred this technique. Although carbon dioxide continues to be used as the main mobile-phase component in nearly

every SFC application, these new instruments are often used with binary or ternary mobile phases, sometimes near or even below what are considered critical conditions. Composition gradients have also become the most popular means of mobile-phase strength programming with the newer packed-column SFC instruments. These practices now blur the distinction between SFC and liquid chromatography (LC), particularly when high-temperature LC is considered. We will not tackle the issue of these distinctions in this review, but will simply recognize that current GC and LC equipment is not capable of practicing SFC (or near-critical fluid chromatography, subcritical fluid chromatography, enhanced-fluidity LC, etc.) without the addition of some means to maintain or control the mobile-phase pressure over the entire length of the column. Therefore, for the foreseeable future, SFC and its close cousins will continue to be practiced separately from GC and LC despite the absence of theoretical boundaries between the techniques.

Growth in supercritical fluid extraction (SFE) has occurred even faster in this time period. There are two main contributing factors. First comes concern over the relatively large volume of solvents required with conventional liquid-extraction methods. We continue to see rising solvent acquisition and disposal costs, limited availability or outright disappearance of some solvents, and regulatory restrictions in some locations on the generation of laboratory waste. The second major growth factor is the exciting new SFE instrumentation. Both highly automated systems and new, low-cost, entry-level systems have appeared recently, accompanied by a vigorous, free-for-all competition among the equipment producers.

This critical review resumes from our last review (1) and covers the literature reported in *Chemical Abstracts* in 1992 and through October 1993. In addition, we have included recent papers published in *Chromatographia*, *The Journal of Chromatographic Science*, *The Journal of High Resolution Chromatography*, *The Journal of Microcolumn Separations*, and *The Journal of Supercritical Fluids* through their October 1993 issues, which were not yet abstracted in *Chemical Abstracts*. (*Chemical Abstracts* has been nearly three years behind, at times, in abstracting some of these journals.) Several noteworthy general references to analytical supercritical fluid technologies will be useful to those new in the field (2-6). Although we have generally omitted articles not readily available through technical libraries worldwide, we recognize the many recent efforts in Japan to broaden the use of SFC and SFE and call attention to one Japanese language review (7).

The papers we have chosen to include are distributed among various topical headings. Most of the papers covering more than one heading are referenced only once in the place where we judged them to fit best. Therefore, we recommend readers

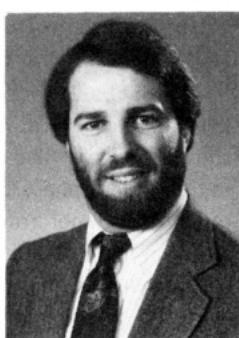
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scanning for specific topics (for example, SFE Extracting Fluids) to also look at related topics (SFC Mobile Phases) to find all the pertinent references.

## BEHAVIOR OF FLUID MIXTURES AND PHYSICOCHEMICAL MEASUREMENTS

The strength or polarity of a supercritical fluid can be enhanced by the addition of another component, or perhaps two or more additives. The strength of the mixture is often difficult to model, but Ekart et al. developed a chromatographic technique to measure cosolvent effects such as hydrogen bonding, charge-transfer complex formation, and dipole/dipole interactions (8, 9). Van Alsten and Eckert also determined that solvent effects are more important than solute structure and that polar and hydrogen-bonding modifiers can have large effects on selectivity (10). Unfortunately, not just the strength, but also the phase behavior must be understood when mixed fluids are used as a chromatographic mobile phase or as an extracting medium. Phase behavior is considerably more

complicated for mixtures than for pure materials, especially near critical conditions.

In SFE, whenever liquids are extracted directly, it is essential to maintain a phase separation. In SFC, we generally want to operate the column under conditions such that the mobile phase remains in a single phase at all times. Even when a neat fluid, such as CO<sub>2</sub>, is used as the mobile phase in SFC, a mixture is formed at least temporarily when sample is injected. If the temperature in the injector is different from that in the column (assuming the pressure is approximately the same in both places), phase separation may occur in one place or the other as the injection solvent passes through the system. This may prove advantageous or deleterious, depending on exactly what happens and what was intended. Thus, understanding the phase behavior of appropriate binary, ternary, or higher mixtures is essential for successful SFC and SFE.

The engineering literature provides a starting point for finding information regarding mixture phase behavior, but published studies often do not cover the full range of composition required by chromatographers. For example, phase equilibria experiments were recently reported for CO<sub>2</sub> and anhydrous milk fat (11), steroids (12), and food additives (13). Suleiman et al. developed an experimental technique using packed-column SFC and evaporative light scattering for the measurement of phase equilibria of heavy paraffins in CO<sub>2</sub> (14). Gurdial et al. determined the critical loci for CO<sub>2</sub> mixed with acetone, 2-propanol, C<sub>1</sub>-C<sub>6</sub> n-alcohols, and C<sub>5</sub>-C<sub>10</sub> n-alkanes, but only up to 7 mol % of the additives (15). While this information is very useful, it is clearly not sufficient for many analytical purposes, for example, when parameters are set in SFC when modifier concentrations are desired higher than the ranges reported or for understanding mass transfer during sample injection into CO<sub>2</sub> streams when one of these solvents is used to dissolve a dilute sample.

Recent studies, as well as work in progress, are aimed at developing the needed understanding. For example, Page et al. studied the phase behavior of CO<sub>2</sub> and propylene carbonate mixtures in a variable-volume view cell and showed clearly how ignoring the phase separation may harm chromatographic results (16). This group later examined tri-n-butyl phosphate/CO<sub>2</sub> and acetone/CO<sub>2</sub> mixtures with the same technique (17). Page et al. then developed a rapid method based on laser-light scattering to map the phase behavior of chromatographically important mixtures (18). Page et al. summarized all of the available phase behavior of chromatographically important solvent/CO<sub>2</sub> mixtures, indicating that sufficient information for successful SFC exists for only five solvent/CO<sub>2</sub> combinations (19). Chester and Innis introduced a rapid method to determine the boundaries of the two-phase mixture region (in pressure and temperature dimensions) using chromatographic equipment (20). So, while some experimental work is being performed to understand complicated, special mixtures, SFC and SFE could clearly benefit from a better and more complete understanding of simple, commonly encountered mixtures. Once the phase information is adequately compiled, simplified chromatographic and extraction methods will surely follow, utilizing the safe zones in phase space for the particular technique and fluid mixture.

Modeling of phase behavior may prove to be useful as an adjunct to empirical testing. For example, Kao et al. developed an equation-of-state analysis for water/surfactant/supercritical fluid mixtures (21). Mishra et al. modeled phase behavior of CO<sub>2</sub> and fatty acid esters (22). You et al. applied a nonrandom lattice-fluid treatment to mixtures including fatty acids and triglycerides (23). Yu et al. examined and modeled ternary mixtures of CO<sub>2</sub>/methyl oleate/oleic acid (24).

Caballero et al. computed binary interaction parameters for solid/supercritical fluid equilibria (25). Krishna Sastry and Mukhopadhyay modeled solvent clustering of small solvent molecules around large solutes (26). Additional solubility studies were reported by Kwan and Mansoori (27) and Ashour and Hammam (28).

Roth reviewed the use of SFC for the determination of diffusion coefficients and thermodynamic quantities (29). Rao et al. modeled solute/modifier interactions for the SFE of fragrances (30). Madras et al. measured solubilities in supercritical fluids by determining the weight of material deposited on activated carbon in a supercritical flow stream (31). Zehnder and Trepp measured mass-transfer coefficients and equilibrium solubilities for two-phase systems involving supercritical CO<sub>2</sub> (32). Staby and Mollerup measured mutual solubilities of 1-pentanol and supercritical CO<sub>2</sub> (33). Yokoyama et al. described an internal reflectance FT-IR cell designed for measurements in supercritical fluid solution (34).

## SUPERCritical FLUID CHROMATOGRAPHY

Several recent reviews on SFC may be of interest, particularly to newcomers in the field (35–39). In addition, all workers in the field should take notice that IUPAC has accepted a Unified Nomenclature for Chromatography, which defines terms and symbols for all types of chromatography, including SFC (40, 41).

**SFC Theory and Fundamental Measurements.** The separation efficiency generated within a given time depends on the rate of solute diffusion in the mobile phase. The diffusion rate may undergo a large change if a modifier is added to the mobile phase, especially if the temperature and pressure are near the critical values of the main mobile-phase component. Shenai et al. measured diffusion rates in neat CO<sub>2</sub> and with 5 mol % methanol added, noting that hydrodynamic and hard-sphere theories cannot account for the effects observed (42). Huetz and Klesper examined the effects of linear velocity, pressure, and temperature on efficiency using a mobile phase of *n*-pentane modified with methanol (43). They examined, among other things, the dependence of diffusion on the varied parameters. Renn and Synovec reported that high-speed size exclusion chromatography (SEC) is possible at high temperatures due to mobile-phase viscosity reduction (and improvements in solute diffusion coefficients) (44). The low viscosity of supercritical fluids, and the low resulting pressure drops compared to liquid mobile phases, allows very long columns to be used. Berger reported achieving 220 000 theoretical plates in packed-column SFC by connecting 11 conventional LC columns in series (45). Janssen et al. compared the theoretical influence of pressure drop on efficiency in packed and open-tubular columns (46). They

noted that a significant pressure drop causes spatial variations along the column in velocity, local capacity factor, and local plate height. Blumberg also considered the effects of velocity variation on column efficiency, concluding that little loss of efficiency should occur within common experimental parameters (47).

Volpe and Siouffi described several shortcomings in GC, LC, and SFC, pointing out that SFC is hindered by a lack of understanding of retention (48). Perhaps not enough effort is being spent to understand fundamentals, with most workers in the field preferring to charge ahead using SFC empirically to solve analytical problems. However, several groups are actively engaged in developing fundamental understanding of the retention process.

Morishita et al. described the retention characteristics of SFC stationary phases in a fashion similar to the McReynolds approach in GC (49). Nishikawa investigated retention of organophosphorus pesticides on five open-tubular stationary phases and found good correlation between retention and a parameter described as the inorganicity/organicity ratio (50). Karlsson et al. modeled retention of lipids on coupled open-tubular columns from retention measurements made on single columns (51). Giddings et al. found that the stationary phase had very little effect on the separation of *n*-butyl acrylate-methyl methacrylate copolymers and a polysulfide elastomer when pressure programming was used in open-tubular SFC (52). Solubility was controlling the separation in these cases.

Smith et al. tested the retention of homologous alkyl aryl ketones and several aromatic compounds by cyano-bonded silica stationary phases, with and without methanol modifier in CO<sub>2</sub> mobile phase (53). The primary interactions appeared to involve silanol groups and were greatly reduced with the addition of methanol. Berger and Deye found that retention of several polar solutes was directly proportional to the surface area of a diol stationary phase in packed-column SFC (54). Sakaki studied the retention of β-carotene and fatty acid methyl esters on octadecylsilane (ODS) and NH<sub>2</sub> columns with both CO<sub>2</sub> and N<sub>2</sub>O mobile phases (55).

Petersson et al. developed models for predicting retention time and peak width in unprogrammed (56) and temperature- or density-programmed (57) open-tubular SFC. Roth developed and tested a statistical thermodynamic treatment of retention in SFC accounting for stationary-phase swelling (58, 59). Roth also examined the apparent linearity of van't Hoff plots ( $\ln k'$  vs  $1/T$ ) in constant-density SFC and showed that they are of little practical use in determining solute transfer properties (60). However, Roth does maintain that SFC can be developed for making reliable thermodynamic measurements (58) and reviewed the use of SFC in obtaining physicochemical information (29). Afrane and Chimowitz modeled adsorption equilibria involving supercritical fluids (61).

Zhang et al. studied the effect of pressure drop on retention and showed that retention can be characterized by a hypothetical zero-pressure-drop system at a density equal to the temporal average density of the actual system (62). Poe modeled retention and efficiency in open-tubular SFC, accounting for temperature, density, pressure drop, and stationary-phase swelling. The work indicates the existence of a second maximum in resolution at high velocities and

suggests the possibility of very rapid SFC separations (63). Janak et al. reported an improved method for on-column measurements of stationary-phase swelling, and found significant contributions to solute band broadening from the stationary phase (64). Goel and Beckman modeled the swelling of cross-linked silicones by CO<sub>2</sub> using a mean-field-lattice gas model (65). Yan and Martire extended the statistical thermodynamic lattice model (66), studied the retention of polycyclic aromatic hydrocarbons (PAHs) on a liquid crystal stationary phase, and found and explained the correlation of retention with minimum area (a shape selectivity parameter) for isomeric PAH molecules (67). They also studied the retention of PAHs in isotropic stationary phases where they found no shape selectivity (68). Reister et al. studied open-tubular column hold-up time by two methods and then calculated outlet densities as a function of inlet pressure and temperature (69). Kueppers et al. calculated the free volume of pure and mixed mobile phases, found a correlation between free volume and SFC retention, and described the results of composition programming with CO<sub>2</sub> and methanol for the separation of polystyrene oligomers (70). Pfund and Cochran estimated chemical potentials of solutes in supercritical fluid mixtures using scaled particle theory (71). Pfund et al. also studied methanol aggregation in CO<sub>2</sub> and ethane (72).

**Stationary Phases and Columns.** SFC continues to be practiced with both packed and open-tubular columns. Poole et al. reviewed SFC stationary phases (73).

Open-tubular SFC columns resemble their GC counterparts except the inside diameter is typically smaller and the stationary phases must be immobilized. The work to develop new polymers suitable for stationary-phase films seems aimed at some specific applications. The most significant of these is size/shape separations, which we will address shortly. Janak et al. immobilized poly(methyl-3-propylthiol)siloxane on open-tubular columns by cross-linking, and evaluated the stationary phase for SFC (74). They also oxidized the films in situ to the disulfide and sulfonic acid forms and then loaded the modified films with silver ions. The silver-ion-modified sulfonic acid film was found useful for the separation of fatty acid methyl esters according to their unsaturation. Malik et al. developed nine new cyanobiphenyl-substituted polysiloxane stationary phases and evaluated them using both open-tubular GC and open-tubular SFC (75). Rotzsche et al. increased the capacity of open-tubular columns by using adhesive to immobilize stationary phase to the tube walls (76).

LC columns are most often used in packed-column SFC. Rotzsche et al. produced column packings by immobilizing a silicone rubber mixture to support particles (77). Funkenbusch et al. prepared carbon-clad zirconium oxide particles (78). Demirbueker and Blomberg packed columns with silica-based ion exchange particles, treated the columns in situ with a potassium permanganate solution, and then used the columns successfully for separations of triglycerides (i.e., triacylglycerols) from vegetable oils by degree of unsaturation (79). The same group also investigated packed-microcolumn argentation SFC for the analysis of triglycerides (80). Ibáñez et al. cross-linked silicone (SE-54) onto silica particles, packed 0.5-mm inside-diameter (i.d.) columns up to 50 cm in length and evaluated them using SFC (81, 82). Earlier we mentioned

the example of multiple coupled LC columns to achieve high efficiency (45). Malik et al. developed a CO<sub>2</sub> slurry method to pack fused-silica columns up to 10 m long with LC packing materials (83). The authors reported theoretical plate counts as high as 240 000.

Several major efforts have taken place (or are still underway) to develop size/shape-selective stationary phases for SFC, and especially chiral stationary phases. Pesek and Williamsen reviewed the use of liquid crystal stationary phases in LC and SFC (84). Five other reviews address the use of SFC in the separation of enantiomers (85–89).

Fujimoto et al. studied the influence of column temperature and pressure on solute-planarity selectivity of encapsulated and polymeric ODS-modified silica stationary phases, using CO<sub>2</sub> as mobile phase in both liquid and supercritical states (90). The morphology of the polymeric phase was significantly influenced by temperature and pressure and produced superior planarity selectivity using the liquid form of the mobile phase (at lower temperatures). Nishikawa separated enantiomers of pyrethrins using Pirkle-type stationary phases with both sub- and supercritical mobile phases (91). Again, subcritical conditions (below the critical temperature) produced better results than supercritical conditions and required shorter analysis times than conventional LC. Zhou et al. cross-linked OV-225-L-Val-*tert*-butylamide to open-tubular columns, evaluated the film for the separation of amino acid enantiomers by GC and SFC, and found better selectivity with SFC due to the lower temperatures compared to GC conditions (92). Johnson et al. synthesized eight (1*R*-*trans*)-*N,N'*-1,2-cyclohexylenebis(benzamide)oligo(dimethylsiloxane) copolymers and found several useful for the SFC separation of enantioergic diols (93). Petersson et al. found, in the evaluation of one of these phases by both GC and SFC, that SFC can produce higher resolution because of its lower operating temperatures (94). Bruegger and Arm found that residual silanol groups on the supporting silica may reduce enantioselectivity of bonded (*R*)-*N*-(1-phenylethyl)-*N'*-(propylsilyl)urea (95). Lou et al. separated enantiomers derived from phenylalanine by SFC on an open-tubular column coated with poly[(cyanoethyl)vinylsiloxane], L-Val-*tert*-butylamide (96).

Much interest exists in the use of carbohydrates as chiral stationary-phase components. Kaida and Okamoto used phenylcarbamates of cellulose and amylose, and the 4-methylbenzoate of cellulose, as stationary phases (97). The phenylcarbamates provided less resolution in SFC than when used in LC, but SFC conditions produced better resolution than LC for the 4-methylbenzoate of cellulose. Javancz et al. mixed cellulose-based chiral materials with achiral polymers, coated open-tubular columns, and evaluated the columns with GC and SFC (98). SFC proved superior. Bradshaw et al. developed a number of new cyclodextrin–oligosiloxane copolymers, produced open-tubular columns, and used them successfully with a variety of chiral solutes (99, 100). Schmalzing et al. (101) and Jung and Schurig (102) evaluated an immobilized polysiloxane-anchored permethyl- $\beta$ -cyclodextrin (Chirasil-Dex) under SFC conditions. Separations of a variety of racemates were accomplished, including ibuprofen, ketoprofen, norgestrel, and hexobarbital. Two of the racemates successfully separated could not be separated with GC conditions. Once again, lower temperatures produced

better results. Armstrong et al. reported a simple method to immobilize a cyclodextrin-based stationary phase on the walls of fused-silica tubing and then used the columns successfully in capillary electrophoresis, GC, and SFC (103).

**Mobile Phases. Dangers of Nitrous Oxide.** Carbon dioxide, either neat or with some modifier added, continues to be the most widely used mobile phase in SFC. It has a great number of advantages, including abundance in high purity, low cost, and a proven safety record. However, these advantages, and particularly the safety aspects, do not necessarily translate to all other fluids used in similar applications. There have been several accidents involving the use of nitrous oxide in SFE systems (for example, see refs 104 and 105). We feel that the use of N<sub>2</sub>O should be stopped not only in SFE, but in SFC as well—it is simply too risky to combine organic material (including the sample, its solvent, and the stationary phase) with a strong oxidizer at elevated temperature and pressure. Any SFC research requiring N<sub>2</sub>O should be limited to open-tubular systems (due to the extremely small volumes required) and performed with appropriate precautions. And, even though Knowles and Richter safely used N<sub>2</sub>O with 1% methanol and tetrahydrofuran modifiers (106), the possibility of a severe accident, we feel, is too great to warrant this practice except under the strictest of safety conditions, if at all.

**Safe Alternatives.** Xenon has been used as a substitute for CO<sub>2</sub>, especially when on-line spectroscopic detection is desired. Jenkins et al. recently compared CO<sub>2</sub> and Xe as mobile phases for SFC/FT-IR and found them to be very similar with respect to their temperature and density effects on the IR spectra (107). Chlorodifluoromethane (Freon-22, a hydrochlorofluorocarbon) has also been investigated as a mobile-phase alternative. Yeo et al. (108) and Ong et al. (109) recently reported that it greatly shortens retention times of phenols and that 11 phenols could be separated using 5% Freon-22 as modifier in CO<sub>2</sub>.

A popular alternative approach is the use of modifiers to improve the strength of CO<sub>2</sub> for specific solutes. It is still widely accepted that, in most packed-column SFC experiments, modifiers in low concentration in CO<sub>2</sub> primarily modify the stationary phase. Cocks and Smith evaluated various diols to dynamically modify silica packings and produce stationary phases resembling diol-type bonded phases (110). Pyo et al. used a saturator column for introducing either water or methanol modifier into CO<sub>2</sub> streams and monitored the composition with an amperometric microsensor (111, 112). Page et al. produced a linear water concentration gradient in CO<sub>2</sub> by programming conditions on a saturator column (113). Oudsema and Poole compared formic acid and formamide as modifiers in CO<sub>2</sub> (114). Both are suitable with flame ionization detection, and they provide somewhat complementary properties. Formamide was particularly well suited for eluting tertiary amines from silica-based columns. Crow and Foley also examined the use of formic acid as modifier (115). Karlsson et al. found formic acid to provide little benefit (in a ternary mobile phase also containing methanol) for the elution of acidic solutes from a cyanopropyl stationary phase (116). However, they found that adding citric acid significantly improved the solute peak shapes. Lesellier et al. examined the effects of modifiers in subcritical fluid chro-

matography using CO<sub>2</sub> as the main mobile-phase component and achieved separations of seven carotenoids in one-third the time required by LC (117). Lesellier et al. tested 16 modifiers in the separation of carotenes by subcritical fluid chromatography and found behavior similar to nonaqueous reversed-phase chromatography (118). Tchapla et al. showed that nonaqueous reversed-phase chromatography parameters could be converted to SFC with the use of CO<sub>2</sub> in the mobile phase, achieving a significant decrease in the analysis time (119).

## SFC INSTRUMENTATION, TECHNIQUES, AND PERFORMANCE

**Pumping.** In the past, the majority of SFC systems contained only one pump. A saturator column could be used to introduce modifier (111, 113), or a modified mobile phase could be mixed prior to its introduction to the pump. This could be done either in the laboratory or with the purchase of premixed mobile phases in cylinders. Schweighardt and Mathias examined the composition of tanks of premixed mobile phases as a function of the mass removed and found that the composition changes as the tank contents are depleted (120). This is in agreement with known phase behavior. Two-pump packed-column SFC systems, with one pump dedicated to CO<sub>2</sub> (or the main fluid) and the second pump dedicated to either a neat or mixed modifier, are growing in use (for example, see refs 121 and 122). Furthermore, if pressure control is actively maintained at the column (or detector) exit, then the inlet flow rates can be selected completely independently from the pressure. Thus, modifier can be blended volumetrically with the main fluid with such a two-pump system by control of the pumping rates. Hirata et al. described a method to reproducibly blend modified mobile phases using a single pump (123). Carbon dioxide flow from the pump was split to provide flow to displace modifier from a reservoir (which was the loop of a six-port valve). Then the streams were recombined. Mixing ratios were controlled by adjusting restrictions in the parallel flow paths.

**Sample Introduction.** There are many opportunities for improving SFC injection over current common practices and for gaining understanding of the mass transfer occurring during the SFC injection process. The practice of SFC could benefit greatly if injection procedures were widely available to increase the effective injection volumes in both packed-column and (especially) open-tubular SFC. Furthermore, workers that are unable to achieve SFC precision on a par with GC and LC are often not aware of problems with their injection procedures. Reviews by Kirschner and Taylor (124) and Greibrokk (125) summarize packed-column and open-tubular SFC injection methods used by researchers.

Injection in packed-column SFC has largely been practiced analogously to LC. However, Oudsema and Poole developed a procedure to remove the injection solvent as vapor from a precolumn, prior to solute transfer to the analytical column (126). This procedure allows injection volumes up to 100 μL on small-bore (typically 1-mm-i.d.) packed columns.

Injection in open-tubular SFC is trickier than with packed columns because of the small dimensions encountered. A 1-μL sample volume will completely fill a 0.5-m length of 50-μm-i.d. tubing. However, Bouissel et al. showed that if

the sample solvent is water (which is a very weak solvent under the conditions used), injections up to 1  $\mu$ L can be easily performed—solutes simply focus on the stationary phase, largely unaffected by the flood of liquid (127). However, whenever a strong solvent is used to dissolve the sample (which occurs most of the time in SFC), inlet flooding can be a major source of band broadening. Chester and Innis described the transfer of injection solvent and solute from a room-temperature injection valve to an oven-temperature SFC column (20). A phase separation of the CO<sub>2</sub>/injection solvent mixture is desirable and may occur, depending on conditions. The authors showed how and when phase separation occurs and how to accomplish direct injection volumes up to 1  $\mu$ L onto 50- $\mu$ m-i.d. columns using a retention gap without solvent venting.

Koski and Lee reviewed solute focusing techniques useful for injection in open-tubular SFC (128). Koski et al. showed how the addition of a small-inner-diameter precolumn could improve solute focusing when using a solvent-vent injection technique (129). Koski et al. described a solvent-venting injector using a short packed column as the solute focusing device, allowing injections up to 3  $\mu$ L onto 50- $\mu$ m open-tubular analytical columns (130). Berg et al. developed a solvent-venting technique combined with gas purging, allowing volumes up to 50  $\mu$ L (131). Hirata et al. described a system relying on a dilution chamber and venting (132). The authors demonstrated the transfer of 100  $\mu$ L of liquid to an open-tubular column. Koski et al. described a solid-phase injector capable of injecting any volume, limited only by the patience of the operator (133). This device is similar to the falling-needle injector used in GC, in which a drop of sample solution is placed on the tip of a needle and the solvent is evaporated. Successive drops can be added and evaporated to build up to the desired effective injection volume. Cortes et al. (134, 135) and Campbell et al. (136) developed an approach capable of transferring 100  $\mu$ L of liquid to open-tubular SFC columns. Variations of their apparatus can be operated either as an SFC injector or as an LC/SFC interface. The device is composed of a series of valves, restrictors, and vents. Open-tubular SFC, performed with this injection approach, achieved better than 0.2% RSD for analyte at the 1 ppm level.

**Other Instrumentation and Performance Issues.** Klesper and Schmitz (137) and Schmitz and Kueppers (138) reviewed the use of gradients of all sorts (e.g., temperature, pressure, composition, etc.) in SFC. Kueppers et al. examined the influence of velocity and multigradient programming on chromatographic efficiency, concluding that a pressure or density program needs a simultaneous negative velocity program to maintain column efficiency (139). Chester and Innis performed open-tubular SFC with positive linear temperature programming at constant density (140). The results using nonpolar columns resembled GC except that chromatograms were uniformly compressed to lower elution temperatures as the experiment was repeated at higher density. Very large selectivity shifts were observed with polar columns as the density was changed. Koizumi and Suzuki described an SFC system using pressure and composition programming (141). They used dual flame-photometric detection for organosulfur compounds and had no interference from the modifiers.

Commercial open-tubular SFC equipment providing pressures higher than 415 atm (1 atm equals 0.101 32 MPa) is not available in the United States. Chester and Innis reported that the use of pressures to 680 atm and temperatures to 240 °C can triple the analyzable molecular weight range of some samples compared to results using commercial SFC instruments (142).

Van Puyvelde et al. reported a technique to determine optimum density programs in SFC (143) and also described an optimization approach for gradient programming in SFC (144). Foley and Crow described a multiparameter optimization approach for SFC (145).

Flow restrictors are still required and widely used to couple SFC columns to lower pressure detectors, such as flame-based detectors and mass spectrometers. Pinkston and Hentschel compared the performance of tapered, integral, and frit restrictors in open-tubular SFC (146). All three performed well at low pressures for weakly retained solutes, but the integral restrictor performed best for strongly retained materials eluting at pressures above 400 atm. The frit restrictor condensed solutes under these conditions and was useless, while the tapered restrictor allowed a rapid increase in mobile-phase velocity with increasing pressure, causing a loss in resolution. Vejrosta et al. described and tested a new type of restrictor for SFC—a glass, multichannel flow restrictor incorporating parallel flow paths (147). Clark and Jones described a fused quartz union using adhesive for coupling open-tubular SFC columns to restrictors (148).

Greibrokk reviewed multidimensional systems involving supercritical fluids (149). Xie et al. demonstrated open-tubular SFC/SFC with determinations of cholesterol, retinoic acids, and a digitalis-like factor, each from biological samples (150). The LC/SFC work of Cortes et al. was described earlier (134–136). Moulder et al. coupled a microcolumn SEC to an open-tubular SFC in a two-dimensional fashion (151). Liu et al. described instrumentation for two-dimensional open-tubular SFC/GC using a thermal desorption modulator as the interface, and applied it to group separations of PAHs (152). Robinson, Tong et al. described a unified approach to sequential GC/SFC on the same column (153, 154) and later improved band broadening through optimization (155).

Cole et al. systematically studied common and unique derivatizing agents applied to SFC (156). Larger blocking groups can be used successfully in SFC than is possible in GC because volatility of the derivative is not a concern if the derivative is soluble in CO<sub>2</sub>. Walther et al. used a new chiral reagent, (*S*)-Trolox methyl ether, to produce and separate diastereomeric esters of enantiomers of aliphatic alcohols (157).

**Detection.** From its inception, versatile detection has been one of the great strengths of SFC. In one form or another, workers have interfaced most detectors designed for GC or for LC with SFC. Some of these mobile-phase detector combinations have proved extremely useful. For example, the flame ionization detector (FID), traditionally a GC detector, provides universal detection for organic compounds which would typically require LC analysis, where universal detection is less straightforward. Also, the ease of removing most mobile phases used in SFC has simplified its interfacing

with spectrometric detection, such as mass spectrometry (MS) and Fourier transform infrared spectrometry (FT-IR). The diversity of the detection options in SFC is reflected in the publications described below. Two reviews touch directly on detection in SFC (158, 159).

**Flame-Based, Electron-Capture, and Photoionization Detection.** The most commonly used detector in open-tubular SFC is the FID. Yang and Baumann found that, with respect to detector base current, the optimum distance from the tip of the flow restrictor to the FID flame tip was 5–7 mm (160). The behavior of a variety of more selective detectors traditionally employed in GC was also investigated. The flame-photometric detector (FPD) was studied by Dachs and Bayona (161). They used a combination of simplex and factorial design approaches in their optimization of the FPD for the detection of organotin compounds in SFC. They found the detection limit for tributyltin chloride to be approximately 40 pg (as tin). Campbell and Richter (162) and Ashraf-Khorassani and Levy (163) studied the thermionic ionization detector (TID, also known as the nitrogen–phosphorus detector or NPD). Both groups studied the selectivity of this detector in SFC. Shirota et al. used a derivatization scheme including the formation of methoximes to yield N-containing derivatives of oligosaccharides (164). They then used a TID in the N mode for detection after SFC. Jew and Richter used the TID for the analysis of halogenated compounds in SFC (165). Detection limits were in the low-picogram range and selectivity was in the  $10^4$ – $10^5$  range versus hydrocarbons.

Howard and Taylor (166, 167) and Sye et al. (168) studied the use of the sulfur chemiluminescence detector in SFC. Howard and Taylor demonstrated that the greater flow of CO<sub>2</sub> mobile phase in packed-column SFC, as well as the presence of methanol modifier in the packed-column mobile phase, did not quench the chemiluminescent species to a greater degree than that experienced in open-tubular SFC (167). Sye et al. found minimum detectable quantities of probe analytes to lie between 20 and 30 pg of S (168). Yarita et al. described use of an electron-capture detector (ECD) for the selective detection of polychlorinated biphenyls (PCBs) in environmental samples (169). The baseline current of the ECD was affected by the flow rate of CO<sub>2</sub>. Electronic compensation was used to eliminate the drift. This same baseline rise of the ECD with flow rate was observed by Tarver and Hill, as discussed below (170). Moulder et al. evaluated the use of the ECD in SFC using agrochemicals as probe analytes (171). They found the detector worked better with packed capillaries than with conventional packed columns. Onuska and Terry evaluated the photoionization detector for packed-column SFC (172). The detector they evaluated was complementary to the FID, but was not suitable for trace analysis due to its large cell volume.

**Evaporative-Light-Scattering Detection.** Universal detection of organic analytes with the FID has been one of the hallmarks of SFC operated with pure CO<sub>2</sub> mobile phase. In packed-column SFC, a polar organic modifier must generally be added to the mobile phase to elute polar analytes. The FID cannot be used with most modifiers. Therefore, a number of groups have used the evaporative light scattering detector (ELSD) for universal detection in packed-column SFC. Members of one group, Dreux and Lafosse, reviewed the

potential of the ELSD for detection in SFC and LC (173). This same group described the use of the ELSD for pharmaceutical analysis (174). They found a narrow range of response factors for a variety of excipients and actives, which is promising for the quantitation of unknowns. This group also used the ELSD to study ethoxylated alcohol and poly(ethylene glycol) samples (175) and cosmetic waxes (176). They found advantages in the packed-column SFC separations over those achieved in LC and GC, respectively. Morin-Allory and Herbreteau used the ELSD to compare the retention behavior of sugars in SFC and HPLC (177). Upnmoor and Brunner studied various parameters affecting the response of the ELSD, such as the nebulizer gas flow, the evaporator temperature, and the percentage of methanol modifier in the mobile phase using squalane (178) and squalane and glucose (179) as probe analytes. They found that the ELSD was applicable over a wide range of conditions. Demirbuker et al. devised a miniature ELSD for packed-capillary-column applications (180). They used it to study the argentation SFC of triglycerides.

**Mass Spectrometric Detection.** While universal detection by FID or ELSD is extremely useful in many applications of SFC, especially those involving screening of unknowns, these detectors provide relatively little information about the nature of the analytes detected. Spectrometric detection provides additional information on this nature, as well as the capacity for universal, specific, and sensitive detection. These attributes generally justify the added complexity and cost of these detectors. Perhaps the premier member of this class of detectors is the mass spectrometer. Progress in SFC/MS was reviewed by Pinkston (181).

The goal of three publications was to reduce the complexity and cost of SFC/MS, and thus to make it more widely available. All three aimed to accomplish this by making use of simpler, less expensive mass spectrometers. Pinkston et al. described the combination of SFC with a Paul-type ion trap using an external ion source and a quadrupole mass filter “ion tube” (operated in the rf-mode only) (182). Detection limits in the low femtomole range were measured for low molecular weight probe analytes. Despite the use of the external ion source, designed to reduce the influence of the mobile phase on the operation of the ion trap, the performance of the instrument degraded at high mobile-phase flow rates. Murugaverl et al. interfaced a benchtop mass spectrometer to SFC using a slightly modified GC/MS interface (183). Library-searchable electron ionization spectra of thermally labile pesticides were obtained. Detection limits were in the low-nanogram to -picogram range in full-scan mode. Pinkston et al. also described the off-line combination of SFC with plasma desorption mass spectrometry (184). While the reproducibility and limits of detection were relatively poor, the technique allows straightforward access to MS data to users not equipped with on-line SFC/MS.

A number of groups discussed the coupling of SFC to mass spectrometry using atmospheric pressure chemical ionization (APCI). This approach is promising as it allows chromatograph and mass spectrometer to operate more independently than in other interface/ionization arrangements. Arpino et al. reviewed previous interfacing attempts and pointed to the promise of APCI, as well as direct electrospray, for SFC/MS

(185). Matsumoto et al. interfaced packed-capillary-column SFC to APCI using a nebulizing interface originally developed for LC/MS (186). They optimized and evaluated the interface using a variety of oligomeric and nonpolar analytes. Tyrefors et al. paid particular attention to the design of their interface probe for SFC/APCI-MS (187). The probe held a constant temperature, to within 1 °C, over its entire length. The corona-discharge ionization was shown to be independent of mobile-phase flow, and the detection limit for trilaurin was 10 pg in the selected-ion-monitoring mode. Fujii described lithium ion attachment for ionization of SFC effluents at atmospheric pressure (188). This soft ionization process produces primarily Li<sup>+</sup> adduct ions.

While not operating at atmospheric pressure, the thermospray (TSP) interface is well suited for packed-column SFC/MS. Scalia and Games optimized various operational parameters of the TSP interface, such as temperature, repeller voltage, and discharge conditions, using bile acids as probe analytes (189). In other developments related to SFC/MS, Kalinoski and Hargiss investigated the low-energy collision-induced-dissociation (CID) spectra of nonionic surfactants introduced to the ion source using an open-tubular SFC system (190). They found the various classes of nonionic surfactants exhibited characteristic CID fragment ions, which depended greatly on the structure of the surfactant hydrophobe. Pinkston and Bowling discussed their use of cryopumping to improve SFC/MS performance at high mobile-phase flow rates, as well as their investigations into the nature of electron ionization in SFC/MS (191).

***Ion Mobility Detection.*** The ion mobility detector (IMD) is an atmospheric pressure relative of the time-of-flight mass spectrometer. Ions generated from the analytes migrate under the influence of an electric field against a countercurrent flow of gas. The arrival times of the ions at the end of the migration space are recorded. The group led by Hill has pioneered the use of the IMD in all types of chromatographies, including SFC. The versatility of the IMD is illustrated in a review by Hill and McMinn (192) and by the articles by Hill et al. (193, 194). Most modified CO<sub>2</sub> mobile phases and alternate chlorofluorocarbon mobile phases had little effect on the positive and negative reactant ion pattern (193). Hill and Tarver illustrated the potential utility of SFC/Fourier transform IMD with a wide variety of applications (195). Finally, Tarver and Hill compared the electron-capture detector to the IMD, operated in the negative ion mode, for the detection of a variety of halogenated and high-electron-affinity analytes (170). The baseline signal of the ECD rose with increasing mobile-phase (CO<sub>2</sub>) flow, while the baseline signal of the IMD was independent of flow, illustrating a significant advantage of the IMD for the selective detection of electron-capturing analytes.

***Fourier Transform Infrared Detection.*** The structural information provided by Fourier transform infrared is often complementary to that provided by other spectrometric detectors. For example, structural isomers are often difficult to distinguish using mass spectrometry. Yet the IR spectrum of these isomers may differ significantly, allowing straightforward identification by FT-IR detection. Two primary means of interfacing SFC to FT-IR have been used: the flow-cell and the solvent-deposition approaches. Each has its own

set of advantages and limitations. Most of the publications abstracted during this period which discussed SFC/FT-IR were overview articles. Most described both approaches (196–199). The reviews by Fujimoto and Jinno (197) and by Bartle et al. (199) cover an impressive range of applications.

Articles by Griffiths et al. (200, 201) focus primarily on the advantages of the solvent-deposition (mobile-phase-elimination) approach. The analytes are deposited on a moving support, which then passes through a tightly focused IR beam. Since the deposited analyte remains on the support, areas of interest may be revisited, and the signal may be averaged for a longer period of time for improved signal-to-noise ratio (S/N). Sheu et al. described an off-line interface for SFC/FT-IR using a cooled, computer-driven collector drum (202). Articles by Belz (203) and Taylor and Calvey (204) focus on flow cell SFC/FT-IR. This approach has often been hampered by poor S/N. Jenkins et al. described a much improved flow cell for open-tubular SFC/FT-IR (205). They measured an approximately 25-fold improvement in limit of detection.

***Plasma-Based Detection.*** Plasmas are used for selective detection of SFC effluents as a means of generating both photons (atomic emission (AE)) and ions (MS). The union of chromatographic methods, including SFC, with plasma AE was reviewed by Uden (206) and by Carey et al. (207). Carey and Caruso specifically reviewed SFC detection using plasma-AE or plasma-MS (208). Luffer and Novotny focused on microwave-induced-plasma (MIP) AE detection of SFC effluents in their review (209), while Jinno (210) and Jinno et al. (211) focused on inductively coupled plasma (ICP) AE detection.

Dowling et al. (212) and McCleary et al. (213) described new torch designs for MIP-AE detection of SFC effluents. Dowling et al. demonstrated that the “concentric dual-flow torch” has a variety of advantages over conventional torches for GC and SFC detection (212). The torch was used for the detection of silicon-containing compounds after open-tubular SFC. McCleary et al. characterized a demountable torch with tangential flow pattern (213). The torch allowed MIP-AE to be used as a selective detector for packed-column SFC. Luffer and Novotny derivatized a variety of analytes to form cyclic boronate esters (214). They then evaluated an MIP for selective detection of boron. The advantages of the He-MIP for nonmetal detection in SFC were reviewed by Webster and Carnahan (215). In a separate publication, these authors described a moderate-power He-MIP-AE for SFC detection (216). The moderate-power plasma did not suffer from the reduction in signal at high SFC flow rates seen in earlier, low-power-plasma experiments. The authors also described spectral regions where CO<sub>2</sub> or N<sub>2</sub>O interfered. Long et al. investigated a He-MIP-AE system for the detection of metals and nonmetals after SFC (217). Pressure programming, even at packed-column flow rates, had little effect on the plasma. They also demonstrated a 5 order of magnitude linear range with detection limits from subnanogram to low-nanogram per milliliter levels for nonmetals.

Caruso and co-workers published extensively on the use of ICP-MS and MIP-MS for selective detection after SFC. Vela et al. reviewed the use of these methods for element selective detection in gas, liquid, and supercritical fluid chromatographies (218). Vela and Caruso described the determination

of organotin compounds by open-tubular SFC/ICP-MS (219). Detection limits were in the hundreds of femtograms. Olson and Caruso investigated the use of He-MIP-MS for the determination of halogenated compounds, using halogenated naphthalene derivatives as probe analytes (220). Detection limits were in the low-picogram range, with a linear range of 3 orders of magnitude (50 pg–50 ng). Finally, Carey et al. investigated the potential for multielement detection in SFC using ICP-MS (221). They compared the performance of time-resolved acquisition (selected-ion monitoring) of ion current signals to single-ion monitoring for organolead and organomercury compounds. Detection limits were in the low-picogram range for both methods, with single-ion-monitoring detection limits being approximately 3–5 times lower for the probe analytes.

**Ultraviolet Absorbance and Miscellaneous Detection.** Ultraviolet absorbance (UV) is the most common method of detection in packed-column SFC using modified mobile phases. The UV flow cell must be designed to withstand much higher pressure than typical in LC for on-line detection. Hirata and Katoh designed a temperature- and pressure-controlled UV cell for open-tubular SFC (222). This allowed the use of UV detection during pressure programming without baseline drift. The cell could also be used for refractive index detection. Sandmann and Grayeski described a sheathing-flow interface for SFC/UV and SFC with fluorescence detection which involves pressure reduction, dissolution of the SFC effluent in a solvent added postcolumn, and detection using a standard, “low-pressure” LC flow cell (223). The system exhibited little contribution to peak width. Meier and Trepp evaluated a calibration method, originally developed for LC, for spectrophotometric detectors in SFC (224). The compressibility of the mobile phase presented special requirements on SFC hardware, and on operational and calibration procedures, to ensure accurate quantitation.

Brown and Roehl successfully coupled a Coulson electro-lytic conductivity detector with SFC for the selective detection of Cl-containing compounds (225). The coupling required no modification to either chromatograph or detector. Detection limits ranged in the tens of nanograms of Cl on column. Both Imasaka (226) and Sin et al. (227) presented overviews of supersonic jet spectroscopic detection. Imasaka described interfaces and procedures for detection following gas, liquid, thin-layer, and supercritical fluid chromatographies (226). Sin et al. focused on the distinct advantages that supercritical fluids bring to supersonic jet spectroscopy, both as a sample-introduction solvent and as a chromatographic mobile phase (227). Hofmann and Spraul patented a sample head for high-pressure, flow-through nuclear magnetic resonance detection following SFC (228). Parrish et al. demonstrated a powerful method of studying biological activity (229). They fractionated a coal tar standard using SFC, directing the fractions to a *Salmonella* microsuspension mutagenicity assay. The coal-tar mutagenic activity was fully recovered in the SFC fractions.

## SFC APPLICATIONS

Because of the historical development of GC and LC, applications most often associated with SFC are those which are difficult to handle using traditional gas or liquid chromatographic methods. SFC should thus be seen as comple-

mentary, not as competitive. For example, SFC's solvating mobile phase may allow elution of less volatile or thermally labile analytes which are difficult to elute in GC. Or the compatibility of the most common SFC mobile phase, CO<sub>2</sub>, with the FID may allow detection of species which are more difficult to detect in LC. The low viscosities of supercritical fluid mobile phases produce low pressure drops allowing longer columns and higher theoretical plate counts than in LC. The applications listed below demonstrate the success of SFC in dealing with these sorts of difficult problems. Most applications involving on-line SFE/SFC are listed separately in the SFE-Coupled Techniques section.

**Foods.** Artz reviewed the use of SFC in the analysis of lipids, glycolipids, fat-soluble vitamins, carotenoids, and steroids in fats, oils, and other food products (230). Borch-Jensen et al. compared the SFC separations of highly unsaturated, fish oil triglycerides using polar and nonpolar open-tubular columns (231). The authors reported that the nonpolar column separated according to carbon number, while the more polar column separated the triglycerides according to degree of unsaturation. We mentioned earlier that Demirbueker et al. had prepared a permanganate-impregnated column and applied it successfully to the separation of triglycerides (79). In addition, they also applied silver- and permanganate-impregnated columns to the separation of unsaturated fatty acid methyl esters from fish oil using SFC (232). Ikawa et al. described an SFC separation of triglycerides (233). Pinkston and Bowling used SFC/MS to characterize the components of olestra, a noncaloric fat substitute (234). An interface modified for higher temperature operation and additional vacuum pumping were required for the components eluting near the end of the pressure program. We also cited earlier several examples involving the separation of carotenoids (55, 117, 118). Lesellier et al. reviewed LC and SFC separations of carotenoids (235).

Morin et al. separated polymethoxylated flavones with packed-column SFC using methanol-modified CO<sub>2</sub> and UV detection (236). They reported that SFC was significantly faster than LC. Morin-Allory and Herbreteau analyzed monosaccharides and polyols by LC and SFC (177). Mills and Jefferies used SFC to determine PCBs in milk (237). Snyder et al. successfully determined tocopherols by both GC and open-tubular SFC (238). GC analysis required derivatization of the samples, but SFC did not.

**Natural Products, Including Oils and Waxes.** SFC has been used extensively for the characterization and analysis of natural products. Foley and Crow (239) and Lubke (240) provided wide-ranging reviews of these applications and of the potential of SFC in this area. Huh et al. used SFC for the analysis of polyprenols in *Ginkgo biloba* leaves (241). The method does not require the extensive cleanup required for previous LC methods, and a C<sub>120</sub> isoprenolog was reported which was not detected by previous methods. Li et al. described the determination of panaxadiol and panaxatriol in *Radix notoginseng* and *Radix yunnanbaiyao* by open-tubular SFC (242).

Demirbueker et al. used a variety of analytical methods for the characterization of triglycerides in *Aquilegia vulgaris* (80). They found argentation-packed-microcolumn SFC to be particularly valuable due to its selectivity for degree of

unsaturation. Brossard et al. used packed-column SFC to study a variety of commercial waxes, including beeswax and hydrogenated jojoba oil, intended for cosmetics (243). They found the overabundance of certain compounds to be harmful to the quality of the wax.

**Pharmaceuticals and Drugs of Abuse.** Publications describing both determinations of active agents and characterization of excipients have appeared, using both packed-column and open-tubular SFC. Peytavin et al. reviewed the potential of open-tubular SFC for the determination of polar pharmaceutical actives (244). Jagota and Stewart published a number of evaluations of open-tubular SFC for the determination of particular types of actives: estrogens (245), diazepam, chlordiazepoxide, and related compounds (246, 247), and nonsteroidal antiinflammatory agents (248). Of three polysiloxane columns evaluated (100% methyl, 30% biphenyl/70% methyl, and 50% cyanopropyl/50% methyl), the cyanopropyl provided best results for the estrogens and the diazepam- and chlordiazepoxide-related compounds, while greater success was achieved with the biphenyl column for the antiinflammatory compounds. Analysis times for various mixtures in all three applications were in the 20–25-min range. The analysis of pharmaceutical excipients is also of great importance in the pharmaceutical industry. Giron et al. used open-tubular SFC with FID to characterize mixtures of mono-, di-, and triglycerides in a number of excipient mixtures (249).

Speed of analysis is an advantage of packed-column SFC. Several authors have exploited this attribute for the analysis of pharmaceutical agents. Scalia and Games used packed-column SFC/UV for the determination of free bile acids in pharmaceutical preparations (250). Once optimized, the separation was at least 8 times faster than previous methods. Gyllenhaal and Vessman used packed-column SFC to determine omeprazole (251). They used methanol containing 1% triethylamine as modifier and found an NH<sub>2</sub>-modified-silica stationary phase to provide the best selectivity and peak shape. The separation of omeprazole and its analogs was complete in less than 10 min. Lafosse et al. discussed the use of packed-column SFC-ELSD with modified CO<sub>2</sub> for pharmaceutical analysis (174). The uniformity of response factors suggested that this combination is suitable for the quantitation of unknowns in stability studies. Siret et al. found unusually good enantioselectivity in separations of β-blockers by packed-column SFC (252).

Preparative-scale SFC holds great promise for “high-value-added” products, such as pharmaceuticals. These advantages were discussed by Johnson and Blum as they presented their use of SFC for the purification of tritium-labeled drugs (253). The technique generated little hazardous (in this case, radioactive) waste, and no evaporation or freeze-drying of the mobile phase was required.

Other publications dealt with controlled drugs or drugs of abuse. MacKay and Reed discussed a variety of applications in this area using open-tubular and packed-column SFC, and SFC/MS (254). Edder et al. used SFE and open-tubular SFC for the extraction and determination of basic drugs of abuse (255).

**Other Materials of Biological Importance.** Xie et al. (256) and Markides (257) reviewed bioanalytical uses of SFC. Open-tubular SFC-FID is a powerful tool for the analysis of

triglycerides, as demonstrated by its use for that purpose in a variety of application areas. Gruber and Rohrbasser used SFC-FID for the characterization of blood-plasma triglycerides (258). A well-deactivated ODS-silica column allowed the determination of cholesterol and cholesterol esters with unmodified CO<sub>2</sub> and simultaneous FID and UV detection (259). Underivatized polar analytes are generally separated in SFC using packed columns and polar modifiers. For example, Camel et al. separated underivatized amino acids using a pyridine (or ethylene glycol)/methanol/water/triethylamine modifier for CO<sub>2</sub>, a diol-bonded silica column, and evaporative-light-scattering detection (260). While the chromatographic conditions were not strictly supercritical, the advantages associated with SFC were preserved in these impressive separations. In a similar fashion, Scalia and Games determined conjugated bile acids using a cyanopropyl-bonded-silica column, UV detection, and methanol-modified mobile phase (261). The method was applicable to human duodenal bile samples. Young and Games used both open-tubular and packed-column SFC for the study of *Fusarium* mycotoxins (262).

Xie et al. described the use of multidimensional open-tubular SFC/SFC for a variety of bioanalytical applications (150). Two interfaces were evaluated, using either flow- or rotary-valve switching.

**Enantiomeric Separations.** The low temperatures (leading to a more ordered stationary phase) and high diffusion coefficients associated with SFC provide special benefits for enantiomeric separations. Petersson et al. developed a multivariate optimization approach for the separation of two enantiomers on a chiral stationary phase in open-tubular SFC (263). The objective of the method was to first achieve a desired resolution and to then minimize retention time without decreasing resolution. Fuchs et al. described a pilot-plant-scale separation of phosphine oxide enantiomers using subcritical CO<sub>2</sub>/ethanol as mobile phase (264). The 60-mm-i.d. column provided up to 510 mg/h of one pair. Optical purity was greater than 95%. Siret et al. separated enantiomeric β-blockers by packed-column SFC, as mentioned above (252). They postulated that CO<sub>2</sub> plays more than a passive role in the separation, forming a complex with the 1,2-amino alcohol moiety of the drug. The complex enhances enantioselectivity. Sakaki and Hirata used a more traditional approach in resolving secondary alcohol enantiomers by SFC (265). They derivatized the alcohols (to form diastereomers) and evaluated three achiral stationary phases. Underivatized polar compounds are difficult, if not impossible, to chromatograph with pure CO<sub>2</sub>. This makes the enantiomeric separation of phenylalanine, DOPA, and related compounds by Lou et al. remarkable (96). As described earlier, they used an open-tubular column coated with a cross-linked poly[(cyanoethyl)-vinylsiloxane], L-Val-*tert*-butylamide.

**Oligomeric Mixtures, Polymers, and Polymer Additives.** The characterization of oligomeric surfactants which do not contain chromophores has been a mainstay of SFC-FID since its inception. A number of publications dealt with this important area of application. Silver and Kalinoski compared high-temperature GC and SFC of ethoxylated alcohols (266). They found that both techniques had advantages and limitations. High-temperature GC provided greater resolution, but

discriminated against higher molecular weight components. SFC did not require derivatization, but did not fully resolve the latest eluting components. Lu et al. used open-tubular SFC to characterize and quantitate propoxylated glycerols and the ester of propoxylated glycerol with oleic acid (267). Wang and Fingas described their use of open-tubular SFC to characterize ethoxylated octylphenol (Triton-X) nonionic surfactants (268). They found simultaneous pressure and temperature programming enhanced the resolution of the later eluting oligomers. They verified their results by comparison to results obtained with LC. The authors published a similar study of the SFC and HPLC separations of ethoxylated nonylphenols (269). Jenkins et al. discussed a variety of applications of analytical supercritical fluid technology, including the use of SFC/FT-IR in the characterization of oligomeric surfactants (270).

The application of SFC to oligomer analysis is not limited to surfactants. Raynor and Bartle reviewed applications of SFC to the characterization of mixtures important in the coatings industry (271). They covered mixtures, such as polyisocyanate curing agents, which were separated using open-tubular SFC and pure CO<sub>2</sub>, as well as more polar mixtures, such as epoxy resins, which were separated with packed-column SFC using modified CO<sub>2</sub> as mobile phase. Simonsick and Litty used SFE/SFC for the characterization of a variety of polymeric samples used in automotive coatings (272). SFC data on methyl methacrylate was complementary to previous data obtained using spectrometric methods. Ute et al. used semipreparative SFC (10-mm × 250-mm packed column) to isolate isotactic and syndiotactic oligomers of methyl methacrylate with degrees of polymerization ranging from 19 to 29 (273). Liebman et al. used SFC and SFE/SFC to characterize coatings designed for composite material fabrication (274). Jacobs et al. studied the retention behavior of homologous 4-*n*-alkoxy-4'-cyanobiphenyl liquid crystals by packed-column SFC/UV (275). Pasch et al. separated poly(1,3,6-trioxocane)s using SFC (276, 277). A single chromatogram revealed both molecular weight and functional group distributions. The ability of SFC to resolve individual end groups in oligomeric mixtures was seen by Schmitz and Gemmel as a powerful tool for monitoring the preparation of macromonomers (278). They demonstrated the concept for styrene and 2-vinylnaphthalene oligomers with a variety of end groups.

SFC has not only been used for characterizing oligomeric mixtures, but has seen increasing use in the analysis of polymer additives, often in combination with on-line SFE, as described later (279, 280). Off-line, packed-column SFC was used by Hunt et al. to characterize poly(vinyl chloride) additives extracted using SFE (281). Berg et al. used open-tubular SFC to determine polymer additives which had migrated from polypropylene to an aqueous acetic acid solution (a food model) (282).

**Fossil Fuels.** The attributes of SFC match well with the challenges provided by mixtures derived from fossil fuels—usually complex and nonpolar. Correspondingly, this area has been a traditional area of application for SFC. The use of SFC/FT-IR for the characterization of coal extracts was reviewed by Griffiths et al. (283). The greatest number of publications in the fossil fuel area involves hydrocarbon-

group separation. These separations are of great importance in the petroleum industry and are quickly and easily performed by SFC. Ashraf-Khorassani et al. (284), Andersson et al. (285, 286), and Fraile and Sanchez (287) all described the use of short, packed columns for quickly separating saturated compounds, alkenes, and aromatics by SFC. Andersson et al. used multiple packed microcolumns (cyano-modified silica, silica, and argentation, in one instance (286)). Analysis times were less than 8 min in a two-column system (285). Small-inner-diameter (50 μm), packed-capillary columns allowed direct-fluid-introduction EI SFC/MS to study the separation (286). Fraile and Sanchez optimized the separation of aromatics using a Doehlert experimental design matrix approach (287). Once optimized, total analysis time was less than 10 min. In a related publication, SFC was used to determine the aromatic ring distribution in nearly 50 diesel fuels by Chen et al. (288). The method correlated well with previous methods.

SFC was used in the characterization of asphalts by Bartle et al. (289) and by Barbour and Branthaver (290). Simulated distillation by SFC was found to have advantages over that performed by GC due to the lower temperatures required, as discussed below (289). Barbour and Branthaver used SFC to compare fractions derived from asphalt in different manners (290). Three fractions derived from a single asphalt were similar, but chromatograms of fractions derived from different asphalts were shown to differ greatly. Desbene et al. compared the SFC and GC determinations of transalkylation products in the characterization of the alkyl-chain distributions in heavy-crude-oil fractions (291). A retro-Friedel-Crafts reaction was used to transfer the alkyl chains from their complex aromatic moieties to a simple aromatic acceptor. While GC did not show evidence for chains longer than C<sub>24</sub>, SFC demonstrated that chains at least as long as C<sub>31</sub> were present.

Simulated distillation of petroleum-derived paraffins and waxes by SFC (for which GC has been traditionally employed) was the topic of publications by Thomson and Rynaski (292), Raynie et al. (293), and Mellor et al. (294). The latter authors investigated a variety of programming and stationary-phase options (294). Thomson and Rynaski saw few differences between "Simdist" curves obtained by SFC and by high-temperature GC (292). Analysis temperatures were much lower for the SFC method. Raynie et al. compared SFC, GC, and thermogravimetric methods (293). They found that SFC provided an extension of the simulated-distillation boiling range over conventional methods. An *n*-octylsilane stationary phase minimized the aliphatic versus aromatic discrepancy common to other chromatographic methods.

The analysis of lubricating oil additives is an important and challenging field in petroleum science. Flake proposed a general method for the analysis of these additives using solid-phase extraction followed by SFC (295). Ashraf et al. evaluated open-tubular and packed-capillary-column SFC for the separation of a group of representative lubricating oil additives (296). They found the diol-modified packed-capillary column to be most successful. All the additives eluted with unmodified CO<sub>2</sub>, allowing use of the FID.

**Pesticides and Environmental Applications.** SFC's capacity for low-temperature separations with reasonable efficiency

has resulted in its use for labile pesticides. For example, Murugaverl et al. described a cleanup flow system for on-line SFE/SFC/MS analysis of carbamate pesticides from meat samples (297). The retention behavior of five organochlorine pesticides on two open-tubular and three packed columns was studied by Wencawiak and Schipke (298). Nishikawa published a series of papers dealing with the SFC analysis of pesticides (91, 299–301). The author first described an extensive evaluation of the SFC retention behavior of 14 common organophosphorus pesticides on four packed and five open-tubular columns (299). The most polar pesticides were too strongly retained on the packed columns, but eluted on the open-tubular columns. Nishikawa then investigated the separation of 13 synthetic pyrethroids on four packed and five open-tubular columns (300). All the geometric isomers of all 13 pyrethroids were resolved with polar open-tubular columns. In a later publication, Nishikawa studied the behavior of the pyrethroids on the open-tubular columns in greater detail (301). Moulder et al. demonstrated the use of the ECD for the detection of agrochemicals, including captan and captafol, after SFC (171).

The wide applicability of SFC to environmental samples is illustrated in the impressive results published by Pospisil et al. (302). They eluted over 270 "EPA Appendix VIII and IX" compounds (compounds of regulatory importance in solid waste and ground water) in less than 1 h using open-tubular SFC. The authors listed retention times, response factors, and chromatographic conditions for many compounds. Ong et al. published two studies describing the SFC determination of PAHs (303) and of nitroaromatics (304) extracted from aqueous samples. They used on-line SFE for the former analysis. Yarita et al. determined PCBs from sediment samples using packed-column SFC with electron-capture detection (169). As mentioned earlier, Yeo et al. (108) and Ong et al. (109) used open-tubular SFC and UV detection for the determination of 11 "priority" phenols. Rather than using unmodified CO<sub>2</sub>, the mobile phase most commonly used in open-tubular SFC, they used CO<sub>2</sub> modified with 5% Freon-22.

**Miscellaneous Applications.** A number of publications described the analysis of organometallic compounds by SFC. Laintz et al. used bis(trifluoroethyl)dithiocarbamate (FDDC) chelation for the determination of As and Sb species (305). Detection limits were 7 pg of As and 11 pg of Sb. In a separate publication, Laintz et al. studied the selectivity of FDDC for As<sup>3+</sup> in the presence of a variety of other metal species (306). Fluorinated FDDC showed significant advantages over its hydrogenated form, both in stability constant for the metal and in solubility in supercritical CO<sub>2</sub>. Zhang et al. used a 30% biphenyl/70% methylpolysiloxane, open-tubular column to separate metal acetylacetones by SFC (307). Detection limits for Cu(II), Al(III), and Cr(III) complexes were in the tens of picograms. Laintz et al. compared the separations of trifluoroacetylacetone and thenoyltrifluoroacetone chelates of Cr and Rh using packed-column SFC (308). The behavior of the trifluoroacetylacetone chelates was superior to that of the thenoyltrifluoroacetone species.

Dachs and Bayona optimized flame photometric detection for open-tubular SFC of organotins (161). The limit of

detection for tributyltin was approximately 40 pg of Sn. Vela and Caruso used FID and ICP-MS detection of organotin compounds after SFC (219, 309). SFC-FID demonstrated greater chromatographic resolution than did SFC/ICP-MS, most likely due to fluctuations in the ICP-MS transfer line temperature. However, ICP-MS detection resulted in a 1 order of magnitude reduction in detection limits (hundreds of femtograms) compared to flame ionization detection. Oudsema and Poole used packed-microbore columns, formic acid-modified CO<sub>2</sub> as mobile phase, and the FID for the determination of organotin compounds (310). Detection limits for most species were in the hundreds of picograms.

The separation of amines by SFC has been the topic of some discussion. Berger and Wilson investigated the separation of aromatic amines by packed-column SFC using methanol/isopropylamine-modified CO<sub>2</sub> as mobile phase (311). A diol silica-based column provided impressive separations, while a polymer-based column did not perform as well. Other miscellaneous applications include the SFC separation (and SFE) of fullerenes by Jinno et al. (312). The authors demonstrated that an extracting and mobile phase composed of toluene-modified CO<sub>2</sub> provided an environmentally safer alternative to existing methods.

## SUPERCritical FLUID EXTRACTION

SFE continues its rapid development as an alternative sample preparation method with the general goals of reduced usage of organic solvents and increased sample throughput (via decreased extraction time and fewer sample handling steps). Although parallel developments are being made in engineering uses of SFE, this review is limited to analytical SFE and will cite engineering or physical chemistry literature only when directly relevant to SFE for analytical sample preparation. During this review period, several cursory reviews of SFE appeared which may be of interest to the reader (12, 313–322).

**SFE Theory and Fundamental Measurements.** As applications of SFE develop, a theoretical understanding of SFE is beginning to emerge. Current thoughts regarding theory and models for SFE involve both thermodynamic (e.g., solvation, partitioning, and diffusion) and kinetic contributions. Bartle et al. proposed a model for dynamic SFE which was based on the effect of solubility on extraction kinetics, as well as solute diffusion out of the sample matrix (323). Meanwhile, Pawliszyn presented a kinetic model of SFE which includes several factors (e.g., desorption kinetics, swelling, analyte diffusion in the organic component of the sample, and fluid/matrix distribution coefficients) that could potentially contribute to slow extraction rates (324). He proposed a comprehensive mathematical model. Cotton et al. evaluated the theoretical basis for observed extraction rates (325, 326). They extracted cyclic trimer from poly(ethylene terephthalate) and found that the extraction kinetics fit a model that presumes the extraction is diffusion-limited (326). The variation of diffusion coefficients with temperature displayed Arrhenius-type behavior. They also used a diffusion-limited model to describe the rate of removal of additives from polypropylene as a function of pressure, particle size, flow rate, and temperature (325). The variation of the extraction rate with

pressure and flow rate confirmed the solubility limitation of the proposed model.

Fundamental physical measurements were also made using SFE. Langenfeld et al. determined the densities of three neat fluids ( $\text{CO}_2$ ,  $\text{N}_2\text{O}$ , and Freon-22) and 5 v/v % methanol in  $\text{CO}_2$  over a wide range of pressures (60–600 atm) and temperatures (40–150 °C) using a simple device constructed from an SFE vessel (327). Chen et al. used a MS composition probe coupled with a batch cell to measure high-pressure phase equilibria at temperatures up to 200 °C and pressures up to 600 bar (328). And Maxwell et al. measured the equilibrium solubility of anthracene and *o*-anisic acid in  $\text{CO}_2$  and methanol-modified  $\text{CO}_2$ , using a modified supercritical fluid extractor with pneumatic valves to inject the solute into a receiver as part of the mobile-phase stream (329). They discussed the effects of varying pressure and sample loop volume on the measurements.

**SFE Instrumentation, Techniques, and Performance.** Wide acceptance of SFE will depend on the evaluation of the performance characteristics of the technique and guidelines for method development. In this regard, experimental design strategies can often be useful. Kane et al. employed an experimental design with multilinear regression to examine the relative contribution of the major experimental variables (330). They determined during their application that the density of the extracting fluid had the greatest effect on the solubilization of steroids and analyte transfer from the extraction vessel to the collection device. Bicking et al. also used a linear regression experimental strategy to optimize temperature and pressure conditions for the SFE of hydrocarbons from solid samples and found that a wide range of conditions could be used to obtain high analyte recovery (331). The possible inadequacies of this approach were debated (332, 333). Langenfeld et al. studied the effects of temperature and pressure on the SFE of environmental samples (334). They found that, for PAH and PCB samples, high temperature can be very important to release analytes that apparently have a strong interaction with the sample matrix. Once adequate pressure for analyte solubilization exists, temperature is a more important variable than pressure. This same research group proposed that this approach of increasing temperature to improve extraction kinetics is more effective than increasing solvent strength for heterogeneous environmental samples (335). The effect of a number of experimental variables has been studied by the research group of Furton (336–338). They reported that extraction recoveries correlate directly with extraction temperature at constant density and with density at constant temperature. Variable extraction recoveries were found as a function of extracting fluid flow rate, extraction vessel dimensions, and other factors. Other authors have attempted to combine these factors for method development purposes. Tehrani presented a flow chart to approach SFE method development (339), while Richter et al. discussed the use of extracting multiple samples in parallel in developing SFE strategies (340). Pipkin (341) and Knipe et al. (342) discussed other systematic approaches and considerations in developing SFE methods.

**Instrumentation.** Instrument vendors, as well as those who assemble instruments in their own laboratories, must consider all of the extraction variables discussed above, and

more, to create SFE systems. Among the factors to consider include temperature and pressure, sample volume, analyte collection, modifier (cosolvent) addition, flow/pressure control, and restrictors. New SFE systems were issued patents during this review period (343–345). Notable among these are an extraction system which includes a fraction collector, automatic sample changer, and automatic extraction vessel transfer for serial extraction of several samples (344) and a system comprising a number of extraction modules and sample separation modules (e.g., SEC) connected to a common fluid supply source (345). Wright et al. developed a portable SFE system for the rapid characterization of soils at the field sites (346, 347). The SFE pumping system employed an LC reciprocating pump with cooled pumpheads. Meanwhile, Pare patented a microwave-assisted apparatus and method for the generation of volatiles and supercritical fluids (348).

Perceptions of the differences between commercially available SFE systems can impede the general acceptance of the technique. King et al. demonstrated that optimized extraction procedures developed on their laboratory-assembled extractor can be directly translated to commercial systems (349). They developed methods for the separation of incurred organochlorine pesticides from coextracted lipid material, total oil extraction from soybean flakes, and the extraction of various pesticides from wheat and successfully transferred these methods to four different commercial instruments. They achieved excellent agreement between results obtained on each instrument for a particular method. Meanwhile, Lopez-Avila et al. tabulated the characteristics and performance of four commercial instruments for SFE of environmental samples (350). They presented perceived advantages and shortcomings. The authors summarized that each system was easy to use but, in their opinion, required skilled operators. Finally, Hurst and Martin discussed the need for automation of new analytical technologies, including SFE (351).

**Extraction Vessels.** Generally, cylindrical extraction vessels are used for SFE and their performance is not questioned. Artz and Sauer developed an improved micro extraction vessel for the on-line SFE/SFC of food and lipid samples (352). They evaluated quantitative performance of the new vessel design at conditions from 100 to 500 atm and 40 to 80 °C. Hedrick et al. demonstrated a special extraction vessel for the extraction of substituted phenols directly from water samples (353). Below 300 atm, they reported that extraction recovery paralleled  $\text{CO}_2$  pressure. Above 300 atm, extraction yield declined with pressure. Hedrick and Taylor also used this same approach for extracting organic bases from aqueous solution (354). Bases with lipophilic moieties (e.g., triprolidine, sulfamethazine, and caffeine) were selectively extracted over picolinic acid, succinonitrile, and nicotine. Liescheski et al. discussed the use of disposable, polymeric extraction vessels and reported fewer potential contaminants with vessels constructed of a new, high-temperature crystalline material than vessels made from poly(ether ether ketone) (355). And Furton and Lin showed that extraction vessel diameter-to-length ratio can have significant influence on extraction efficiency, depending on solute-analyte interactions (356).

**Solute Collection.** Collection of the extracted analyte following SFE is a discrete, yet important, step in the overall

extraction procedure. Significant losses of analyte can occur during this step, leading the analyst to believe that the actual extraction efficiency was poor. During the past two years, significant understanding of the factors influencing off-line (i.e., not directly coupled to the subsequent measurement technique) solute collection has occurred. There are two options for off-line collection: collection in a solvent or trapping onto a solid surface.

Langenfeld et al. studied the parameters affecting solvent collection (357). They reported that good collection efficiencies depend on efficient partitioning of the analyte into the collection solvent. Solvent properties, including polarity and temperature, are more important than solvent volume in obtaining minimal solute loss. Porter et al. also found that collection solvent temperature, as well as volume, influences trapping of the extracted analyte (358). They found that secondary trapping devices do not significantly affect collection efficiency. Their studies were performed with a dual-chamber trapping vial that they patented (359).

Ashraf-Khorassani et al. examined the use of cryogenically cooled adsorbent traps for off-line collection (360). They found that the flow of extracting fluid does not affect collection efficiency, but collection of volatile analytes requires low trapping temperatures (<40 °C). Also important is the solvent used to rinse the analytes from the sorbent bed. Mulcahey and Taylor noted that collection temperature and modifier concentrations are important in trapping efficiency when organic solvent-modified fluids are used during extraction (361). A heated micrometering valve and conventional solid-phase extraction (SPE) cartridges were used for the recovery of trace analytes extracted from liver tissue (362). High extraction flow rates, up to 3–4 L/min expanded gas, could be accommodated by this collection assembly. McNally et al. constructed two SFE systems for routine laboratory use (363). They demonstrated that the restrictor could be eliminated and solutes could be effectively collected during decompression following static extraction. This unique collection method, combining features of solid trapping and solvent collection, was described by Miller, Hawthorne, and McNally (364). They eliminated the restrictor from the extraction vessel and rapidly sprayed the vessel effluent into a solventless test tube following static extraction. They reported efficiencies greater than 95% for a variety of analytes and claimed that recoveries normally exceeded those obtained using dynamic SFE with collection in a liquid solvent.

**Extracting Fluids.** Many of the stated advantages of SFE, such as reduction of organic solvent use, are directly related to the widespread use of CO<sub>2</sub> as the extracting fluid. However, there is some interest in exploring the use of organic solvent-modified CO<sub>2</sub>, alternative fluids, or chemical derivatization, particularly for the extraction of increasingly polar compounds. Wallace et al. reported a method to reduce the levels of contaminants in pure CO<sub>2</sub> (365). They carefully heated the head of their syringe pump to extractively remove contaminants due to the seals and valves of the SFE system. Carbon dioxide purity was also an issue for on-line SFE/GC. Nielsen et al. evaluated several grades of CO<sub>2</sub> available in Europe (317). They concluded that, for trace analysis, no single grade could be recommended. Good-quality CO<sub>2</sub> is available for flame ionization and thermionic detectors, but severe background

contaminations are observed when an ECD is used. Levy et al. studied seven different CO<sub>2</sub> modifiers using on-line SFE/GC (366). For extracting hydrocarbons and PCBs, they reported propylene carbonate and benzene gave the highest extraction efficiencies. They also discussed practical aspects of using modifiers for SFE/GC and studied the same effects with additional CO<sub>2</sub> modifiers (367). Famhy et al. also investigated the effect of CO<sub>2</sub> modifiers (368). They observed swelling of modifier-saturated plant materials and clays with a high-pressure sapphire view cell and determined a relationship between the extractability and swelling of these materials.

A number of alternatives to CO<sub>2</sub> have been examined. Hawthorne et al. compared Freon-22, N<sub>2</sub>O, CO<sub>2</sub>, and methanol-modified CO<sub>2</sub> for the extraction of PAHs and PCBs in environmental samples (369). In each case, the highest recoveries were found with the Freon and the lowest with pure CO<sub>2</sub>, presumably due to the high dipole moment of Freon-22. Carbon dioxide, SF<sub>6</sub>, N<sub>2</sub>O, and SF<sub>6</sub>-modified CO<sub>2</sub> were compared by Levy et al. for on-line SFE/GC of environmental and petroleum samples (370). They noted that different extraction selectivities could be obtained using these fluids and judicious choice of extraction temperature and pressure. Oostdyk et al. discussed the use of CO<sub>2</sub> and N<sub>2</sub>O, neat and with modifier, for the extraction of primary aromatics amines and compared SFE to classical sonication methods (371, 372). The effect of the sample matrix was studied and the greatest extraction efficiencies were observed with N<sub>2</sub>O modified with 1,6-hexamethylene diamine in methanol. Despite other successful uses of SFE using N<sub>2</sub>O, two independent research groups experienced explosions when using N<sub>2</sub>O (104, 105). In both cases, the authors warned against using N<sub>2</sub>O, a strong oxidizing agent, for SFE, even under mild conditions. As we mentioned earlier, we strongly warn against the use of N<sub>2</sub>O in SFC and SFE.

**In Situ Derivatization, Ion Pairing, and Complexation.** Extractions of polar, and even ionic, materials with nonpolar fluids, like CO<sub>2</sub>, are becoming possible through the in situ chemical derivatization of the sample. The approach of Hills and Hill was to use a reactive modifier, hexamethyldisilazane or trimethylchlorosilane, to derivatize the active sites on the surface of the sample matrix, releasing the PAH analytes for extraction (373). The same group also performed initial experiments with silylation, ion pairing, methyl esterification, and ionic displacement for the SFE of 2,4-D from soils (374). Methyl esterification and ionic displacement proved to be the most promising for quantitative extraction. The research group of Hawthorne used chemical derivation of polar and ionic solutes during extraction to alter extractability (375, 376). Trimethylphenylammonium hydroxide and boron trifluoride were used to derivatize and extract herbicides from soil and sediments, microbial phospholipid fatty acids from whole cells, and wastewater phenolics from water and ODS sorbents with greater than 90% recovery. Lee et al. extracted phenols using in situ acetylation with supercritical CO<sub>2</sub>, triethylamine, and acetic anhydride (377). The SFE approach and steam distillation displayed similar results. Methyl esters of seven chlorophenoxy acid herbicides were formed while these compounds were being extracted from soil (378). Trimethylphenylammonium hydroxide, benzyltrimethylammonium chloride, benzyltriethylammonium chloride, and

tetrabutylammonium hydroxide/methyl iodide were studied as derivatization agents.

SFE with ion-pair reagents was used by Field (379) and Fernandez et al. (380). Both groups extracted cationic surfactants from sewage sludge. Field presented a broad range of analyte/ion-pair combinations with derivatization in heated GC injection ports during subsequent analysis (379). Earlier work by Field et al. also demonstrated the quantitative determination of sulfonated aliphatic and aromatic surfactants in sewage sludge by SFE/ion-pair formation, followed by injection-port derivatization GC (381). They reported relative standard deviations of typically 5% for replicate analyses. Venema and Jelink demonstrated the use of reactive additives to the extracting fluid (382). The addition of citric acid to methanol-modified CO<sub>2</sub> during the SFE of calcium montanate resulted in the complexation of the calcium and simultaneous formation of extractable free fatty acids. Liu et al. extracted Cu<sup>2+</sup>, Co<sup>2+</sup>, Cd<sup>2+</sup>, and Zn<sup>2+</sup> with supercritical CO<sub>2</sub> and a complexing agent (383). They modified the extraction vessel with nonmetallic components to avoid coextraction of metal ions from the vessel.

## SFE-COUPLED TECHNIQUES

The direct combination of SFE with other analytical techniques can, in many cases, be a straightforward procedure, giving SFE an advantage over other extraction methods. The on-line coupling of SFE with chromatographic techniques has been reviewed (340, 384, 385).

**SFE/GC.** Much of the development of SFE/GC was done early in the evolution of analytical SFE. Directly coupled SFE/GC has been reviewed (386, 387). Gere et al. accomplished a combination of SFE with GC, though not by direct deposition of the extracted material onto the GC injector, using a robotics approach (388). They applied this approach to the extraction of environmental samples. Lou et al. examined the effects of CO<sub>2</sub> flow rate, interface temperature, and split ratio on the discrimination and reproducibility of on-line SFE/open-tubular GC with a conventional split/splitless injector as the interface (389). The use of a venting valve and a small-diameter LC column was used to couple SFE to GC (390). The system was demonstrated for the ppb analysis of the pesticide chlorpyrifos in grass samples. The research group of Lee used a thermal modulation approach to coupling SFE with open-tubular GC (391–394). The thermal desorption modulator interface is subjected to a thermal pulse to inject a sharp plug of solute onto the GC column for high-speed separation. Fixed-time modulation signals and signal averaging allows rapid screening of volatile compounds. They combined this approach with radio frequency plasma detection for the analysis of environmental samples. Detection limits of 24.8 and 9.2 pg/s for Cl and S, respectively, were obtained.

SFE/GC with AE detection was reported by two groups (395, 396). In the first, halogenated and sulfur-containing pesticides in soils were analyzed. In the second, organotin compounds, some requiring *in situ* derivatization with *n*-pentylmagnesium bromide, were extracted from soil. A 23-factor experimental design was used to study the effect of pressure, temperature, and extraction time on sample recovery.

Johansen et al. applied 20 mL of human milk or 5 mL of plasma onto a sorbent for the analysis of PCBs in the nanogram range using SFE/GC (397). They also selectively removed PCBs from the lipid matrix in different biological species using basic alumina as a selective adsorbent and found that fat content did not seem to influence performance of the system (398). They discussed solute trapping, choice of restrictors, extraction volume, and detection limit. Analyte/matrix interactions in environmental samples were studied by on-line SFE/GC (399). On-line SFE/GC with derivatization was examined by King et al. (400). Triglycerides extracted from seeds were transesterified to the methyl esters *in situ* over a solid catalyst. SFE conditions were chosen so that the methyl esters were selectively eluted from the solid catalyst. A cryotrap/thermal desorption unit external to the GC was used to collect the extracted compounds and introduce them to the GC system in the SFE/GC analysis of volatile oils (401). Murphy and Richter used SFE/GC for the analysis of the pesticide aldrin from several food matrices (402). Using an ECD, they reported a minimum detection level of 0.23 ppb.

**SFE/SFC.** Direct coupling of SFE with SFC has been reviewed, covering theory, interfacing techniques, instrumentation, and applications (403). Many applications of coupled SFE/SFC during this review period concerned polymer additives. MacKay and Smith used the technique to measure thermally sensitive chlorinated organophosphate flame retardants in polyurethane foams (404), while Baner et al. measured CO<sub>2</sub> flow rates during the quantitative SFE/SFC analysis of polymer additives (405). Their apparatus measured the CO<sub>2</sub> concentration in air at the FID and at the split restrictor outlet using electroconductivity principles. Acetic acid-modified CO<sub>2</sub> was necessary for the SFE/SFC analysis of dialkyltin compounds present as stabilizers in poly(vinyl chloride) (406). The effect of temperature on analyte solubility and diffusion through the polymer matrix was studied.

Additional applications covered environmental analysis. Ong et al. reported 91% extraction efficiency for determining PAHs in aqueous environmental samples with microscale SFE/SFC (303). Jinno et al. utilized SFE/SFC with toluene-modified CO<sub>2</sub> for the determination of fullerenes in carbon soot (312). SFE was coupled to a tandem SFC/GC system for the rapid determination of polychlorinated organics in complex matrices (407). Lin et al. studied the influence of various adsorbents as sample matrices on extraction efficiencies (408). They found that extraction efficiencies decreased with increasing surface area and decreasing pore size of the adsorbent. Recoveries of PAHs and PCBs decreased with increasing molecular mass. Meanwhile, Fuoco and Griffiths employed SFE/SFC with FT-IR to determine the effects of pressure, temperature, and time on the extraction efficiency of PCBs in soil samples (409). Mills and Jeffries used an SFE/SFC procedure to determine PCBs in milk (237). Munder et al. directly coupled SFE with open-tubular SFC and three chromatographic detectors for the microanalysis of explosives and propellants (410). Using UV, FID, and ECD, they observed detection limits as low as 100 pg and demonstrated fingerprinting of firearm propellant residues for forensic investigations.

Several other SFE/SFC applications are reported. Berg et al. used an innovative SFE/SFC approach for the analysis of fatty acids and triglycerides (411). They used an immobilized lipase on a catalyst bed to form methyl or butyl esters of fatty acids from triglycerides in edible fat during their on-line procedure. The determination of retinol palmitate and tocopherol acetate in a hydrophobic ointment by coupled SFE/SFC was also reported (412). The analysis employed ethanol-modified CO<sub>2</sub> and UV absorbance at 284. Turmerones and cucuminoids were fractionated from plant rhizomes and separated by SFC in an on-line coupling to SFE (413). The SFC separation of curcuminoids was comparable to the standard LC approach. Murugaverl et al. described a cleanup flow system for on-line SFE/SFC/MS analysis of carbamate pesticides from meat samples (297). Murugaverl and Voorhees also used the sample approach for the trace analysis of pesticides in soybean oil and rendered fats (414). Rocca et al. used on-line SFE/SFC to quickly and efficiently determine an animal feed marker used in a toxicological test (415). Perrigo and Joyst provided an impressive SFC database of over 100 compounds of forensic toxicological importance (416). The authors used both UV and FID detection (in series) and also investigated the use of on-line SFE/SFC. Roston presented a novel application of SFE/SFC for pharmaceutical analysis (417). He extracted a synthetic prostaglandin from a controlled-release drug formulation followed by SFC. The only sample preparation required was weighing the sample into the extraction vessel. As discussed earlier, Liebman et al. used SFE/SFC to characterize coatings designed for composite material fabrication (274). Daimon and Hirata (279) and Ashraf-Khorassani et al. (280) used on-line SFE/SFC for the extraction and separation of polymer additives. The technique was demonstrated with polypropylene samples (279). Hunt et al. evaluated extraction time, temperature, pressure, methanol modifier, and sample particle size for the SFE of additives in poly(vinyl chloride) (281).

**Other SFE-Coupled Techniques.** Other on-line combinations of SFE with analytical methods were demonstrated. Stalling et al. showed the utility of on-line SFE of environmental and biogenic samples and SEC cleanup (418). They determined the efficiency of the SFE/SEC method and studied the effect of extraction fluid flow rate and sample size on peak resolution. Liu et al. measured sub-ppm levels of chlorinated phenols in a variety of solid matrices using SFE coupled online with LC (419). The speed of analysis and selectivity of the SFE/LC approach compared favorably to traditional methods. SFE was combined with flow injection analysis (FIA) using a membrane phase separator to remove CO<sub>2</sub> from the extracted sample (420). The SFE/FIA system was employed for the analysis of chloramphenicol and penicillin G. A high-pressure IR flow cell was used to directly couple SFE with FT-IR without the need for intermediate chromatographic separation (421). Quantification was performed by integrating the IR signal area of the extracted analyte versus time. The detection limit of *n*-tetracosane using this method was reported to be 74 ng.

Finally, SFE was combined with various bioassay procedures. France and King combined SFE with an enzyme assay to screen meat products for pesticides (422). The major benefit of the described approach is that the analyst is not exposed

to organic solvents during the procedure. Similar to the SFC work already reported (229), the use of the *Salmonella* microsuspension assay for the quantification of model mutagens trapped from an airstream was presented (423). Linear dose/response relationships were obtained. Wolfe et al. used SFE for the sample preparation of aqueous pesticide samples for bioassay (424).

## SFE APPLICATIONS

The range of samples for which SFE has been applied continues to broaden. In the previous sections, sample types requiring special techniques, such as in situ derivatization, and samples analyzed by SFE coupled on-line to the analysis technique were presented. Applications of SFE to environmental, food, and polymer analysis have been discussed (425). The growing list of samples extracted using SFE is presented in the following section.

**Fossil Fuels and Environmental Samples.** SFE is apparently gaining its greatest initial acceptance by the environmental community for the extraction of hydrocarbons, PAHs, PCBs, and other compounds from soils, sediments, airborne particulates, and similar samples. Reviews of the SFE approach to environmental analysis have been presented (426, 427). Hawthorne et al. discussed the development and evaluation of off-line and on-line SFE methods for petroleum industry products and wastes (428). Suitable reference materials are needed to establish the validity of new methods, such as SFE. Benner discussed the available Standard Reference Materials developed by the National Institute of Standards and Technology (429). Particulate matter, sediments, and mussel tissue standards were characterized for such contaminants as chlorinated pesticides, PAHs, and PCBs. Myer et al. compared SFE to Soxhlet extraction, with an emphasis on environmental samples, for the sample preparation of herbicides, pesticides, hydrocarbons, waxes, and fats from a number of solid matrices (430). Furton et al. studied a variety of experimental parameters influencing the SFE of PAHs and PCBs from solid-phase sorbents (431). Extraction vessel dimensions, fluid flow rate, adsorbent system used, and analyte molecular weight all had effects on extraction efficiency. McNally et al. presented an in-depth investigation of the influence soil type has on the SFE of moderately polar compounds in soil samples (432). A study on the use of metal-containing selective sorbents for the determination of the constituents of cigarette smoke was performed (433). The sorbents were thoroughly characterized, and blanks observed with SFE were typically cleaner than those obtained with thermal desorption. Griest et al. recovered 2 ppm spikes of chemical warfare agents on soil in less than 2 min using 5% methanol-modified CO<sub>2</sub> at 300 atm and 60 °C (434). Recoveries were higher but less precise than those achieved with microscale ultrasonication. And in one of the few inorganic applications of SFE, Louie et al. quantified the amount of elemental sulfur in coal samples using SFE with GC and AE detection (435). The total amount of elemental sulfur extracted from coal samples was independent of coal particle size. The elemental sulfur values determined by SFE correlated well to the inorganic sulfur fractions, and not the organic sulfur amounts, generated by standards methods.

**Hydrocarbons.** The determination of total petroleum hydrocarbons (TPH) by SFE, often using IR detection, is of great interest. Eckert-Tilotta et al. extracted soil samples contaminated by heavy fuel oil, diesel fuel, light crude oil, gasoline, or kerosene spills using both SFE and Soxhlet techniques followed by either IR or GC-FID (436). Using rather mild SFE conditions, the SFE method compared favorably to Soxhlet extraction with Freon-113, except in some cases where improved recovery of BTEX (benzene, toluene, ethylbenzene, xylenes) was claimed for the SFE approach. The TPH method developed by Lopez-Avila et al. used supercritical CO<sub>2</sub> at 340 atm and 80 °C and compared favorably to the standard Freon-113 Soxhlet technique (437). They evaluated SFE parameters, including pressure, temperature, extraction time, and collection solvent, and conditions for IR determination. The method detection limit was reported as 10 µg/g of sample. Field site evaluations of generator-powered SFE and IR instruments were conducted for TPH extractions (438). Virtually identical results were obtained by field analysis or laboratory analysis of the same sample, with 30-min SFE extractions being in agreement with 4-h Soxhlet extractions.

Two research groups were interested in extracting hydrocarbons from petroleum source rocks. Ashraf-Khorassani et al. reported that SFE of biological markers from sedimentary rock gave greater yields and shorter extraction times than Soxhlet extraction (439). The effect of temperature on the extraction efficiency of biomarkers was also determined. Greibrokk et al. used neat CO<sub>2</sub>, tetrahydrofuran-modified CO<sub>2</sub>, and 2-propanol-modified CO<sub>2</sub> and CS<sub>2</sub> to extract petroleum source rocks (440). With a three-stage extraction, the first extraction was dominated by saturates, the second stage by aromatics, and the third by N-, S-, or O-containing compounds. This method was also useful for the determination of biomarkers. Brooks and Uden used SFE, followed by either microbore SFC or GC/MS, for the extraction and analysis of diesel fuel from soil (441). They reported extraction efficiency of greater than 90% regardless of the organic content of the soil. The adsorptive differences in three clay samples (montmorillonite, kaolinite, illite) were noted in the recovery of diesel fuel from the clays, as determined by SFE and GC (442). However, the high extraction efficiency and short extraction time made the SFE method more attractive than classical methods. Lee et al. spiked crude oils onto soils with organic matter contents of 0.6–30%, artificially weathered the samples, extracted by SFE, and analyzed the extracts by GC/MS (443). They obtained promising results in all cases. Ho et al. presented SPE with supercritical fluid elution and GC/MS determination of semivolatile organic compounds in reagent water, tap water, and river water (444).

**Polycyclic Aromatic Hydrocarbons.** Paschke et al. extracted PAHs and nitrogenated PAHs from diesel exhaust particulates and diesel soot using CO<sub>2</sub>, solvent-modified CO<sub>2</sub>, and Freon-22 as SFE extracting fluids (445). The highest extraction yields were achieved with Freon-22 or with 10% toluene in CO<sub>2</sub>. Burford et al. also compared SFE with methylene chloride sonication for the removal of PAHs ranging from naphthalene to benzo[b]fluoranthene from petroleum waste sludge, urban air particulate matter, and railroad bed soil (446). While performing spike recovery experiments with

the deuterated analogs of the PAHs of interest, they noted significant differences in recoveries of spiked analytes versus the native compounds, demonstrating that spike recovery studies are not valid for developing quantitative extraction methods.

Lopez-Avila and Beckert spiked and extracted sand samples with 36 nitroaromatics, 19 haloethers, and 42 organochlorine pesticides and also extracted a certified reference soil with supercritical CO<sub>2</sub> in a two- or four-vessel extractor to establish extraction efficiencies for samples extracted in parallel (447). They explored variables including flow rate, pressure, temperature, moisture content, extraction vessel volume, extraction time, modifier type, and others. Dichloromethane was used as a static modifier in the SFE of PAHs in soils for routine measurement (448). Within-day and day-to-day reproducibilities of SFE were comparable to conventional methods, and in two samples used in a quality-control program, measured PAH concentrations were similar to those obtained by 11 other laboratories. Meyer et al. performed SFE of PAHs in soils samples containing about 50% carbon content (449). Pyle and Setty presented the use of a copper scavenger column following the extraction vessel to remove elemental sulfur from soils containing high sulfur concentrations during the SFE of PAHs (450). Without the copper scavenger, deposition of elemental sulfur in the restrictor hindered the CO<sub>2</sub> flow. Wenclawiak et al. reported the need to dry soils prior to PAH extraction by SFE (451). Saim et al. used toluene-modified CO<sub>2</sub> to extract fullerene homologs from carbon soot (452). Levy et al. discussed SFE considerations for subsequent GC/MS analysis of PAHs in soils and sediments (453). Ho et al. continued their development of SFE elution of SPE media for aqueous samples containing PAHs, PCBs, organochlorine pesticides, and phthalate esters (454, 455). Using experimental design techniques, they claimed pressure has a statistically significant effect on extraction efficiency, followed by extraction time and temperature.

**Polychlorinated Biphenyls, Dibenzofurans, and Dioxins.** Advances in this application area were reviewed by Greibrokk (456). His review covered SFE of polychlorinated organics from sediments, wastewater sludges, soils, plant and animal tissues, and SPE adsorbents. Kapila et al. reported that the extraction of organochlorine compounds from environmental matrices by SFE is a promising method, but may be limited by the initial instrumentation costs and impurities in commercial fluid sources (457). The selectivity of various adsorbents toward the fractionation of complex organic mixtures consisting of compounds having similar solubility parameters and containing polychlorinated dibenzo-p-dioxins (PCDDs) was examined (458). Florisil, alumina, carbon, ODS, and cyano-modified silica were used and a fractionation scheme employing Florisil was developed. The Florisil cleanup method was applied to the analysis of PCDDs and polychlorinated dibenzofurans (PCDFs) (459). Full-scan mass spectra were obtained on picogram levels of analyte. Aroclors, 17 organochlorine pesticides, 25 organophosphorus pesticides, 43 neutral/acidic compounds, and 16 PAHs were spiked onto sand as the adsorbent and SFE parameters were investigated (460).

Polychlorinated organics in soils were of interest to a number of investigators. A simplex optimization method was used to

optimize conditions for the SFE extraction of PCDDs and PCDFs from soils (461). The optimized conditions were compared to Soxhlet extraction. Van der Velde et al. used SFE to extract PCBs from two soils, with low and high carbon contents, respectively (462). They examined the influence of extraction time, pressure, static versus dynamic extraction, restrictor type, and collection solvent. Liu et al. carried out experiments to optimize the extraction parameters and determine the role of CO<sub>2</sub> modifiers with different sample matrices for the SFE of chlorinated toxins from soil (463). A dynamic tracer response technique was used to measure the adsorption equilibrium and rate of extraction of hexachlorobenzene from soil (464). Methanol modifier in CO<sub>2</sub> had no advantages over pure CO<sub>2</sub>, but increased partition coefficients were found with supercritical ethane. Meanwhile, Moody et al. used bench-scale SFE of 50-g to 5-kg soil samples to study the feasibility of using SFE for remediation of contaminated soils (465). Finally, Onuska and Terry extracted PCDDs from municipal incinerator fly ash (466, 467). Acid treatment prior to extraction was found to provide increased extraction yields (466) and N<sub>2</sub>O-based extracting fluids were also examined. Onuska et al. also compared SFE to Soxhlet extraction for PCBs determination (468). They found greater reproducibility with SFE for the extraction of PCBs from municipal fly ash.

**Pesticides and Herbicides.** Similar to the environmental extractions of soil samples previously discussed is the extraction of soil samples containing agrochemicals. The kinetics of extracting soils spiked with atrazine were studied and fit to the hot-ball model (469). The extraction conditions were predicted from calculated solubilities using the Peng–Robinson equation of state, and satisfactory experimental agreement was achieved. Addition of water to aged soils containing fluometuron was necessary to improve SFE recoveries (470). Carbon dioxide modifiers, temperature, pressure, sample mass, and extraction time were also evaluated, and the SFE procedure was compared to conventional solvent extraction. Howard and Taylor studied the SFE of sulfonyl ureas (471, 472). They extensively considered solid-phase trapping conditions and used methanol-modified CO<sub>2</sub> for the extraction. They immobilized aqueous samples of the herbicides onto SPE media prior to SFE. They also examined the trapping conditions for using SFE to extract thiocarbamates in apples (473, 474). Subsequent analysis was performed by LC with UV or sulfur chemiluminescence detection. Ezzell and Richter also used SFE removal of aqueous pesticide samples trapped on SPE media, with applicability of the method down to the 1 ppb range (475). Snyder et al. compared the SFE of selected organochlorine and organophosphate pesticides to sonication and Soxhlet methods and studied the effect of instrumental parameters and soil matrix on SFE (476, 477). Pesticide recoveries increased with increasing density and pressure, while temperature had little effect on the extraction and even caused thermal degradation of some compounds. Residues of fluazifop-P-butyl and its metabolites were extracted directly from onions without subsequent cleanup steps (478). Quantitative recoveries were achieved, and limits of detection of 0.02 ppm were reported. Carbon dioxide and CO<sub>2</sub> modified with either acetone, ethyl acetate, or methanol were compared in terms of recovery, extraction rate, and matrix effects for

the SFE isolation of organophosphorus pesticides from soil (479). Skopec et al. used methanol-modified CO<sub>2</sub> extraction, followed by GC-ECD, to quantitatively determine organophosphorus pesticides in rice at levels down to 10 ng/g (480).

**Foods and Fragrances.** Since the use of organic solvents and/or high temperatures can be reduced or eliminated by SFE, the technique is also gaining popularity for food and fragrance applications, particularly fats and essential oils. Maxwell et al. described the efficient recovery of volatile nitrosamines from frankfurters, followed by GC with chemiluminescence detection (481). Recoveries ranged from 84.3 to 104.8% for samples spiked at the 20 ppb level. Li and Hartland studied the influence of ethanol and water modifiers on the solubility and selectivity of the SFE of xanthines and cocoa butter (482). Marsili and Callahan compared SFE with an ethanol/pentane procedure for the extraction of carotenes from a number of vegetable samples (483). The SFE method proved favorable in terms of both extraction yield and speed.

**Essential Oils.** SFE of essential oils from a number of plant species has been compared to other extraction methods. SFE was compared to hydrodistillation for the extraction of essential oils from savory, peppermint, and dragonhead (484). A static SFE step with the addition of methylene chloride was necessary prior to dynamic extraction. Greater than 90% recovery of compounds as volatile as monoterpenes was reported and SFE recovered some organic compounds (C<sub>27</sub>–C<sub>33</sub> odd-chain *n*-alkanes) not extracted by hydrodistillation. Extraction of vetiver oil was compared by SFE and steam distillation (485). Not only was SFE more efficient, but a certain degree of selectivity was observed. That is, at lower pressures sesquiterpene hydrocarbons were selectively extracted, while at higher pressures oxygenated compounds were preferentially extracted. Polesello et al. used SFE for strawberry aroma analysis (486). They found that SFE was more selective than liquid solvent extraction and was better able to recover the “character impact” of the strawberry. Enantiomer ratios of carvone in the essential oils extracted from caraway seeds and spearmint leaves were determined by LC with UV and polarimetric detection (487). The major volatile and semivolatile components of both SFE and methylene chloride extracts of tobacco were characterized by open-tubular GC/vapor-phase IR/MS (488). The stated advantages of SFE for this application were reductions in necessary laboratory space, sample processing time, materials costs, potential solvent residual contamination, and manpower. Verschueren et al. optimized the SFE fractionation of the essential oil and bitter principles (humulones and lupulones) of hops (489). They characterized extracts from different hop varieties by microcolumn LC and by micellar electrokinetic chromatography. Reverchon studied SFE mass-transfer characteristics for the fractionation of the essential oils from the cuticular waxes in marjoram leaves (490).

**Fats and Oils.** A review article summarizes the CO<sub>2</sub> SFE of vegetable and animal fats, including vegetable oils, polyunsaturated glycerides from animal fats, and the fractionation of milk fats and cholesterol (387). Lembke and Engelhardt proposed an SFE method for the determination of the total fat content of meat and cheese products as an alternative to the official German solvent extraction method

(491). Acid treatment prior to extraction is necessary to determine total fat content, rather than just free accessible fat. SFE results agree with Soxhlet methods for the determination of the total oil contents of soyflakes, canola seed, and wet-milled corn germ (492). Precisions between the two methods were comparable. Esquivel and Bernardo-Gil extracted the oil from olive oil solid residues using CO<sub>2</sub> at 12, 15, and 18 MPa and 308, 313, and 318 K (493). The SFE of PCBs in freeze-dried milk resulted in coextraction of the PCBs and milk fat; however, by combining SFE on-line with SFC, analytical selectivity was achieved (237). A simplex optimization procedure was used to determine working conditions for SFE. A study of the separation of biomolecules by SFE focused on the extraction of essential fatty acids from fish oils (494).

**Polymers.** The increased diffusivity of supercritical fluids over liquids, as well as the variable solvating strength, has made SFE attractive for polymer applications. We previously discussed that Raynor and Bartle reviewed the use of SFC and SFE for application to surface coatings analysis (271). Kueppers dealt with the selective extraction of low molecular weight components of poly(ethylene terephthalate) (322). A systematic study of the influence of temperature was performed to develop a model for dynamic SFE which considers the temperature-dependent physical changes in polymers. Pressure and mobile-phase composition were also explored, and guidelines for estimating the optimum temperature for the SFE of polymers were presented. A supercritical analog of temperature-rising elution fractionation was developed and used to characterize the heterogeneity of linear low-density polyethylene (495). Results indicated that the fractions are significantly more homogeneous than the unfractionated sample. Meanwhile, SFE fractionated low molecular weight, high-density polyethylene at different temperatures to achieve molecular weight selectivity (496). Higher temperatures yielded extracts containing higher molecular weights. The highest molecular weight fraction extracted using CO<sub>2</sub> centered around 1500. Ethylene-methylacrylate copolymers were fractionated using supercritical propane, propylene, butane, 1-butene, or Freon-22 (497). Isothermal fractionations with increasing pressure were used, demonstrating that chemical-composition fractionation could be achieved by selecting solvents that preferentially dissolve the nonpolar ethylene-rich or the polar acrylate-rich oligomers. Venema et al. investigated the SFE extraction efficiency of removing caprolactam and its oligomers from nylon-6 using methanol-modified CO<sub>2</sub> (498).

Another area related to SFE characterization of polymers is the extraction of polymer additives. Braybrook and MacKay investigated SFE as a standard test protocol for obtaining additives from polymer matrices (499). The authors analyzed the extracts for *in vitro* cytotoxicity using standard cell culture techniques. A silica column was used to selectively remove additives from all but the lowest molecular mass oligomers of liquid poly(alkylene glycol) lubricants (500). The approach was then used for a stepwise extraction of sorbitan ester formulations to result in a chemical class fractionation.

**Natural Products and Drugs.** One of the interests in natural products analysis is the determination of aflatoxin B1. Engelhardt and Haas noted that with pure CO<sub>2</sub> they could

extract lipid constituents from animal feeds, but required pressures of 820 atm at 40 °C to extract the more polar aflatoxin (501). The use of methanol modifier could extract the aflatoxin at lower pressures, but decreased the selectivity of the extraction. Pressures from 2000 to 15 000 psi and modifier additions of up to 20 v/v % of 2-propanol or an acetonitrile/methanol mixture were investigated for the extraction of aflatoxin B1 from field-inoculated corn (502). An alumina column supercritical cleanup method removed coextracted lipid matter. Intercomparisons were performed to develop procedural quality criteria as a certification exercise for the determination of aflatoxin B1 content in five animal feed reference materials (503).

A great number of other natural product types have also been extracted by SFE. Smith and Burford used cellulose as a model matrix system to study the SFE of typical plant constituents (504). They observed that increased selectivity for polar analytes, such as lactones, was obtained by solute trapping onto silica with selective elution. Methanol-modified CO<sub>2</sub> was used to extract tobacco-specific *N*-nitrosamines onto Tenax GR, followed by thermal desorption GC with a thermal energy analyzer (505). Detection limits of less than 2 ng/g were determined. Freeze-drying was needed prior to SFE for the removal of synthetic organochlorine compounds from aquatic plant tissues (506). The use of in-line Florisil cleanup during the CO<sub>2</sub> extraction resulted in markedly lower recoveries for four of the nine analytes. Vindoline was extracted from leaf tissue, and remarkably, the highest yields were achieved at the lowest temperature (35 °C) and the highest pressure studied (507). The addition of ethanol modifier to the CO<sub>2</sub> extracting fluid did not significantly increase the extraction yields. The fungal metabolite ergosterol was removed from flour, moldy bread, and mushroom samples by SFE with sensitivities of about 0.05 µg/g (508). Pressure, temperature, and solute collection methods were studied for the extraction of the sesquiterpene lactone parthenolide from dried feverfew samples (509). Methanol or acetonitrile CO<sub>2</sub> modifiers gave higher yields with less selectivities, but cellulose or silica trapping columns improved the extract purity. The effects of time, temperature, pressure, decompression rate, and moisture on enzymatic activity were studied, with temperature and moisture increasing the rate of enzyme inactivation (510).

The greatest interest in the SFE of drugs and pharmaceuticals during this review period was concerned with the extraction of drugs and residues from animal feeds. A polar lignan, secoisolariciresinol, and its diglucoside derivative were extracted from flaxseed using CO<sub>2</sub> modified with a tetrahydrofuran/water mixture (511). It was necessary to remove the fatty material from the sample with a preliminary extraction and then apply more rigorous conditions to release the lignans from the cell wall material. Carbon dioxide-, N<sub>2</sub>O-, and methanol- or acetonitrile-modified fluids were compared for the quantitative SFE of pharmaceutical agents spiked into rodent and dog feed (512). Meanwhile, SFE was compared to sonication and Soxhlet extractions for the analytical removal of a corticosteroid from rodent diet (513). The steroid compound was determined at levels of 10 ppm in the rodent diet. Messer and Taylor also investigated extracting a hypolipidemic compound from a drug formulation, from rat

feed, and from feed/drug mixtures (514). They observed high extraction variabilities for the drug formulation, indicating heterogeneity. Bicking used a two-level, two-factor factorial design to determine SFE conditions for the characterization of an amine hydrochloride in avian feed (515). Extraction at low temperature and high pressure gave the highest recoveries and also provided the lowest levels of coextracted nonvolatile material. He reported high recoveries at levels as low as 5 µg/g. The extraction of polar drugs, sulfonamides, was shown to require more rigorous conditions when extracting animal tissues, such as chicken liver or swine muscle, than when extracting from an inert matrix, such as sand (516). Higher recoveries were reported for extracting from the swine muscle than the chicken liver, and the results for determined incurred drug were in good agreement with reference values. Basic drugs of abuse were extracted via SFE and analyzed by open-tubular SFC (255). Aqueous morphine samples were adsorbed onto ODS prior to extraction, which was precisely controlled through a novel restrictor design. Direct extraction of the aqueous sample was compared to indirect extraction of the sample immobilized onto Celite for the determination of active components in a liquid drug formulation (517). Meanwhile, the technique for combining SFE with SPE was pioneered for the removal of pharmaceutical compounds in plasma (518, 519). Following addition of the plasma to the SPE cartridges, the proteins were washed off, and then the drugs of interest were eluted via SFE. Selectivity of the method, as well as accuracy and precision, was reported.

**Miscellaneous Applications.** Perhaps one of the most interesting examples of SFE is concerned with metal-containing compounds. Although the initial reports of solvation in supercritical fluids dealt with metal complexes, little work has been done in this area since then. Lin et al. extracted lanthanide and actinides ions from solid material by supercritical CO<sub>2</sub> containing a fluorinated β-diketone (520), while Simon and Papageorgiou extracted river sediments containing inorganic metal salts and organometallics with supercritical CO<sub>2</sub> or methanol-modified CO<sub>2</sub> (521). In the later study, the presence of water in the sample, methanol modifier, and low pressure favored extraction of metal salts, and organometallics may be extracted by either CO<sub>2</sub> or methanol-modified CO<sub>2</sub>, depending on the polarity of the analyte.

Slack et al. characterized a plastic explosive by CO<sub>2</sub> SFE followed by GC-ECD and GC/MS and compared the SFE extracts to the explosive vapors (522). The nonpolymeric components of aged single-base propellants were extracted with supercritical CO<sub>2</sub> and analyzed with open-tubular GC (523). Kuitunen et al. studied the analysis of chemical warfare agents in soil by SFE and open-tubular GC with flame ionization or nitrogen/phosphorus detection (524). SFE was also used to compare chemithermomechanical wood pulps and Kraft pulps (525). Paraben preservatives in cosmetic products were isolated by CO<sub>2</sub> SFE under optimized time and temperature conditions (526). And the injection molding binder (90% paraffin wax, 5% epoxy, 5% oleic acid) was extracted from Si<sub>3</sub>N<sub>4</sub> ceramics via supercritical CO<sub>2</sub> (527). The extraction data were fit to a shrinking core diffusion model.

## OTHER SUPERCRITICAL FLUID MEASUREMENTS

For emerging technologies such as SFC and SFE to continue their development, practitioners must look beyond the current state of the art to examine other possibilities. Supercritical fluids are being studied and gaining acceptance not only as analytical techniques, but in other fields, including physical chemistry and chemical engineering. We will briefly mention selected measurements with supercritical fluids that may eventually find analytical applicability.

**Reactions.** Brennecke published an extensive review of spectroscopic investigations of reactions in supercritical fluids (528). Dumont et al. reported the continuous synthesis of ethyl myristate by enzymatic reaction in supercritical CO<sub>2</sub> (529). They studied effects of water concentration and fluid flow rate.

**Polymer Applications.** Carbon dioxide and SF<sub>6</sub> were used to isolate thermoplastic waste mixtures by density (530). A cubic equation of state was used to correlate fluid density with temperature, pressure, and fluid composition. Yalpani discussed the use of supercritical fluid CO<sub>2</sub> to modify (e.g., oxidation, phosphorylation) several types of polymers (531). And supercritical fluid fractionations of nonionic surfactants (532) and poly(ethylene glycols) (533) were demonstrated.

**Miscellaneous Supercritical Fluid Applications.** Cholesterol was eliminated from a number of food commodities including liquid egg yolk (534), dried egg (535), and meat products (536). The use of SFE technology for fat reduction was discussed (537). Eggers and Wagner developed a new separation scheme for deoiling highly viscous media, such as soy lecithin, with pure supercritical CO<sub>2</sub> (538). Supercritical CO<sub>2</sub> was used to regenerate activated carbon loaded with high molecular weight organics (539). Experimentally determined isotherms were modeled by the Freudlich equation and the heats of adsorption were measured. Polyunsaturated fatty acids were extracted from fungal samples by SFE with CO<sub>2</sub> with and without ethanol modifier (540). Mass-transfer coefficients were determined and correlated as a function of interstitial velocity. A two-step procedure for extracting flavors from milk fat was presented (541). Gas antisolvent expansion of dimethyl sulfoxide and dimethylformamide with supercritical CO<sub>2</sub> was used to produce biologically active powders of insulin with controlled particle size (542) and a similar approach was used for controlled drug release applications of drug-loaded polymer microspheres and small protein particles (543). A supercritical fluid method for controlling the rate of antibody release from a biocompatible ethylene-vinyl acetate copolymer was developed (544). Isenschmid et al. examined the effect of high-pressure CO<sub>2</sub> on the viability of yeast and microbial cells (545). SFE was studied as a method for producing carbon fibers (546, 547). Tavana and Randolph studied the salting-out crystallization of organic solids from isobaric/isothermal supercritical fluid mixtures (548).

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