1

Historical Development of SFC

OUTLINE

1.1 Physical Properties of Supercritical Fluids

In supercritical fluid chromatography (SFC), the mobile phase is ideally in the supercritical state. The meaning of the word supercritical (literally, above critical) is explained in Figure 1.1. The figure shows a phase diagram for a single (pure) component. Depending on the temperature (T) and the pressure (P), three different states of matter may be distinguished. These are gas (G), liquid (L), and solid (S) states. At the triple point (tp) all three of these phases may coexist. Above the critical point (cp) a difference between gaseous and liquid states can no longer be observed. This region is illustrated in Figure 1.1 by the dashed lines, which defines the supercritical fluid region and the material is referred to as a supercritical fluid (Schoenmakers, P.J. and Uunk, L.G.M., "Mobile and stationary phases for supercritical fluid chromatography," Private Communication.).

The supercritical fluid region is not a fourth state of matter. Crossing one of these dashed lines does not result in a phase change, whereas crossing a solid line does. Both condensation and evaporation are phase changes, during which the physical properties (e.g. density, viscosity, and diffusivity) change abruptly. On the other hand, a gas can also be transformed into a liquid in a manner indicated by the curved arrow in Figure 1.1. During this process, a phase

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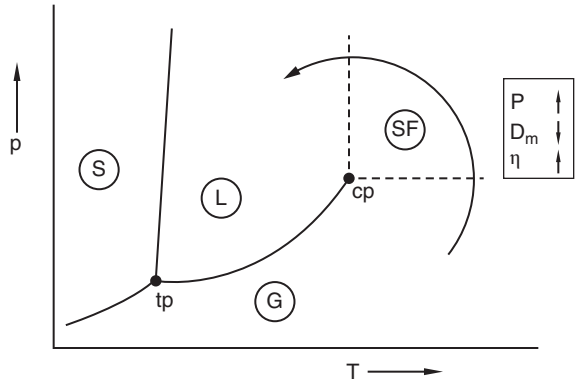

Figure 1.1 Phase diagram for a single pure component, illustrating areas in which solid (S), liquid (L), gaseous (G), and supercritical (SF) conditions occur. tp is the triple point and cp is the critical point. A gas can be transferred into a liquid by following the arrow. In doing so, the density, the viscosity, and the diffusion coefficient change continuously from gas-like to liquid-like values, but no phase change is observed. *Source:* Schoenmakers [1, p. 102].

change is *not* observed; yet, gas is transformed into a liquid. More generally stated, the physical properties of a pure compound show continuous rather than abrupt variations when passing through one of the dash lines.

The region of the phase diagram at temperatures and pressures higher than the critical temperature and critical pressure values was formally (and arbitrarily) designated as the supercritical fluid region by both the American Society for Testing and Materials (ASTM) and by the International Union of Pure and Applied Chemistry (IUPAC) (see Figure 1.2). This designation introduced what appears to be a fourth state of matter, the supercritical fluid. A second designation can be found in Figure 1.3 wherein two subcritical regions are identified along with the supercritical fluid region. Chester has cautioned that this format is an immense source of confusion among novices and even some experts [4]. In this diagram, the supercritical fluid region is formally defined as shown, however the apparent boundaries are not phase transitions, only *arbitrary definitions*.

The literature is full of statements regarding the transition between a liquid and a supercritical fluid phase or between a vapor and a supercritical fluid phase. *This is incorrect* according to Chester. A discontinuous phase change is predicted when the boiling line is crossed, but no discontinuous transitions or phase changes take place for isothermal pressure changes above the critical temperature or for isobaric temperature changes above the critical pressure. *There are no transitions into or out of a supercritical fluid state even though the supercritical fluid region is defined formally* according to Chester.

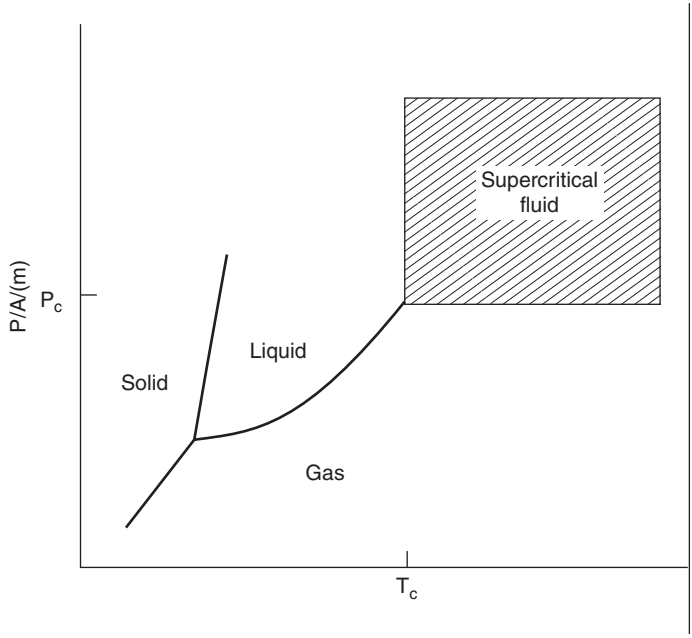

Figure 1.2 Definition of supercritical fluid by American Society for Testing and Materials (ASTM) and International Union of Pure and Applied Chemistry (IUPAC). *Source:* Smith [2]; ASTM [3]; Chester [4, vol. 2, p. 11, figure 2].

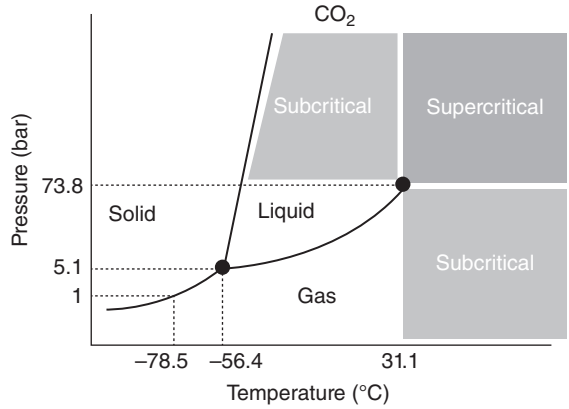

Figure 1.3 Misleading phase diagram for a single component supercritical fluid. *Source:* Laboureur et al. [5]. https://www.mdpi.com/1422-0067/16/6/13868. Licenced under CC BY 4.0.

In other words, it is possible to convert a liquid to a vapor, or a vapor to a liquid, without undergoing a discontinuous phase transition by choosing a pressure/temperature path that is wholly within the continuum. The required path simply goes around the critical point and avoids going through the boiling line. The distinction between liquid and vapor simply ceases for temperatures and pressures beyond the critical point [6]. As stated previously, Figure 1.1 is the accurate depiction of phase behavior. There is no fundamental difference between supercritical fluids and gases or liquids. Rather, a supercritical fluid may best be thought of as a very dense gas!

A more useful description of supercritical fluids for chromatographers is shown in Figure 1.4. In chromatography, multi-component supercritical mobile phases are frequently employed instead of a pure supercritical fluid. It is useful to continue thinking of the fluid phase behavior, but this requires one to expand the phase diagram to include the composition variation possible in a binary mobile phase [7]. Six general types of binary-mixture systems have been defined [8]. Some of the systems have large miscibility gaps rendering them useless for chromatography over much of their composition ranges. Type I mixtures, however, are the simplest and most widely used mixtures in liquid chromatography (LC). These are mixtures in which the two components are miscible in all proportions as liquids [7]. To consider the phase behavior of a

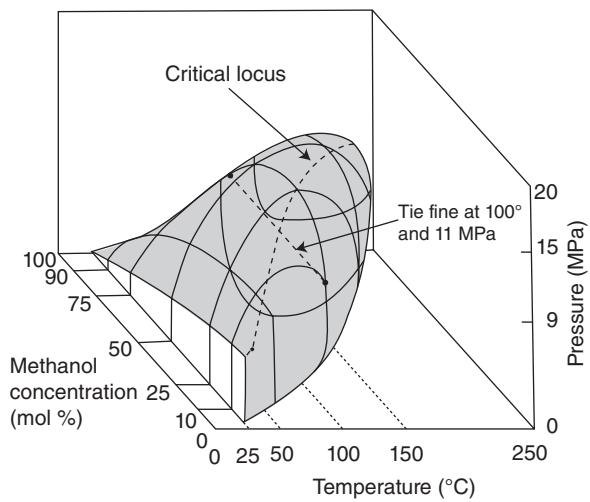

Figure 1.4 Two-phase *l-v* region of a binary mixture is a volume in a three-dimensional phase diagram. The Type I mixture CO_2-methanol is illustrated here. The two-phase region is the shaded interior of the figure. It has been cut off at 25 °C to show the isotherm, but actually extends to lower temperatures. *Source:* Adapted with permission from reference [4]. Reproduced with permission of American Chemical Society.

binary mixture it is necessary to add to the phase diagram a third axis representing the fluid composition. The two components here are arbitrarily called "a and b" except that "a" will be used to designate the more volatile component. The "a and b" choices are restricted to materials that together form a Type I binary mixture [8]. The reader is encouraged to consult reference 8 for a more complete interpretation of these plots.

In other words, there is no narrowly defined supercritical phase [4]. The behavior of a supercritical fluid may be very similar to that of a gas which would be the case just above the horizontal dashed line in Figure 1.1. Such a gas-like supercritical fluid possesses a relatively low density, a low viscosity, and high-diffusion coefficients. Just to the right of the vertical dashed line in Figure 1.1, a supercritical fluid may behave much more like a liquid. Such a liquid-like phase would show relatively high density, high viscosity, and low-diffusion coefficients. The most popular properties of supercritical fluids are listed in Table 1.1.

Whereas the physical properties of a liquid and solid are fixed, the physical properties of a supercritical fluid vary between the limits of a normal gas and those of a normal liquid by control of pressure and temperature as shown in Figure 1.5. Typically, supercritical fluids are used at densities ranging from 10 to 80% of their liquid density and at practical pressures for applications ranging from 50 to 300 atm. Under these conditions, the diffusion coefficients of supercritical fluids are substantially greater than those of liquids. Similarly, the viscosities of supercritical fluids are typically 10–100 times lower than liquids. These more favorable physical properties (as listed in Table 1.1) afford the advantages of supercritical fluids in chromatography and extraction applications.

"Supercriticality" is another term for a fluid that has reached a temperature higher than its critical temperature and a pressure higher than its critical pressure. Although rare, supercriticality exists in nature. For example, the atmosphere of the planet Venus is made of 96.5% carbon dioxide. Figure 1.5 pictorially compares the atmospheres of Venus (left) and Earth (right). At ground levels on Venus, the temperature is 735 K and its pressure is 93 bar. Therefore, in dealing with carbon dioxide, these conditions cause CO_2 to be supercritical on planet Venus.

Table 1.1 General properties of supercritical fluids.

• High diffusivity (gas-like)
• Low viscosity (gas-like)
• Zero surface tension
• Tunable solvent strength
• Nontoxic if CO_2

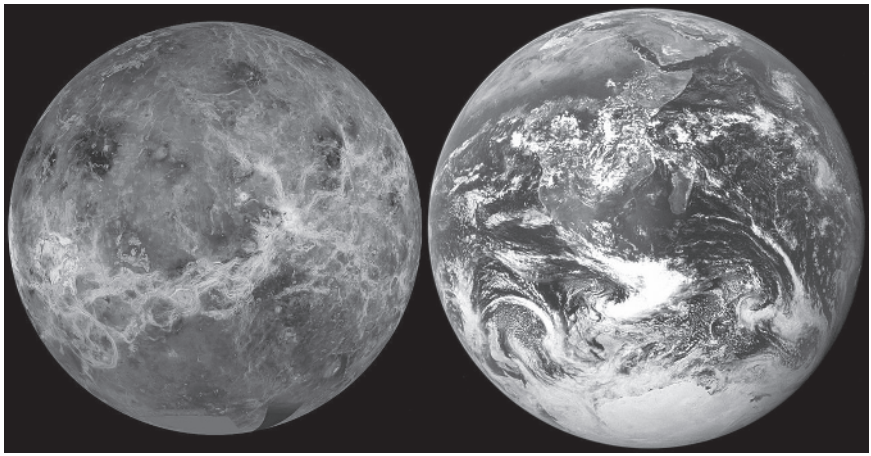

Figure 1.5 Comparison of Venus (CO_2) and Earth (Air) atmospheres. *Source:* Courtesy of NASA.

1.2 Discovery of Supercritical Fluids (1822–1892)

The phenomenon of the "critical state" was first described in 1822 by French engineer and physicist Charles Baron Cagnaird de la Tour when he noted the lack of discontinuity (i.e. disappearance of a meniscus) when passing between gaseous and liquid states in his famous cannon barrel experiment [9]. It was the work of the Irish chemist Dr. Thomas Andrews, Vice President of Queen's College in Belfast, Ireland in 1869 with CO_2, however, which is considered to be the first systematic study of a gas–liquid critical point [10]. It was also where matter was first referred to as a "supercritical fluid." It should be noted, nevertheless, that the general idea of the "critical state" was earlier and independently rumored by Mendeleeff in 1861 while working in Heidelberg with physicist Gustav Kirchoff where he discovered the principle of gases critical temperature. Mendeleeff's work went unnoticed, such that the discovery of critical temperatures is usually attributed to Thomas Andrews. Table 1.2 may be considered to contain a partial listing of the early studies wherein supercritical fluid behavior was demonstrated [11].

In 1879 and 1880, Hannay and Hogarth published the first account of the enhanced solvating properties of supercritical fluids [12] with an experimental apparatus earlier described by Andrews. Hannay's and Hogarth's original belief was that the ability to dissolve solid substances was a unique property of liquids. In their experiments, solutions of colored solids in liquids were heated through their critical points. When the liquids became gaseous, the solids were expected to precipitate and the fluids were predicted to become colorless. In practice, no such precipitation was observed, and the field of supercritical fluid extraction was born.

Table 1.2 Early studies of supercritical fluid behavior.

(1822) First report of supercritical fluid behavior
(1869) First measurement of critical parameters
(1879) First solvation of metal salts by gaseous fluids
(1879) First high pressure expt. via Hg column in mine shaft
(1892) Mercury column as high as the Eiffel Tower

Other investigators made similar observations at an earlier date [13]. In studying the solubility of inorganic salts such as cobalt(II) chloride, ferric chloride, potassium bromide, and potassium iodide in supercritical carbon dioxide, Hannay and Hogarth found a perfect continuity of liquid and gaseous states. Hannay summarized the findings later by stating that: "*The liquid condition of fluids has very little to do with their solvent power, but only indicates molecular closeness*. Should this closeness be attained by external pressure instead of internal attraction, the result is that the same or even greater solvent power is obtained. The gas must have a certain density before it will act as a solvent, and when its volume is increased more than twice its liquid volume, its solvent action is almost destroyed" [14].

Hannay's and Hogarth's experiments were largely based on transition metal salts and supercritical ethanol at temperatures too high to be convenient for a modern lecture demonstration. A lecture demonstration for supercritical fluids involving supercritical ethane with T_c 32.3 °C and blue dye, guaiazulene, has, however, been described for projection of an image of a high pressure silica capillary cell so as to be viewed by a large audience [15]. An excellent review of early studies regarding solubility measurements in the critical region was later provided by Booth and Bidwell [16].

Another informative, pictorial comparison of solvating properties appeared on the cover of Chemical and Engineering News (June 10, 1968 issue) that described the supercritical–liquid–gas inter-relationship (see Figure 1.6). Each of the enclosed glass vessels contained three spheres of unequal density and carbon dioxide. The temperature of the fluid on the far left is above the critical temperature. Thus, with no meniscus the condition was deemed supercritical fluid and at uniform density. Each of the spheres has a different density, thus, the high-density sphere in this bulb sunk to the bottom; while, the lowest density sphere rose to the top. The intermediate sphere density matched the supercritical fluid density and appeared to be suspended in the bulb.

Moving from left to right in the figure, there is a temperature decrease. As evidenced by the cloudiness in the second bulb, the CO_2 is at the critical temperature, and critical opalescence is predicted. At the third bulb from the left, temperature and pressure have decreased further, subcritical conditions exist, and a gas and liquid phase now appear. Two spheres floated on the liquid while the highest density sphere sank to the bottom. The temperature of the fourth bulb was thought to be lower than that in bulb #3. All three spheres floated on

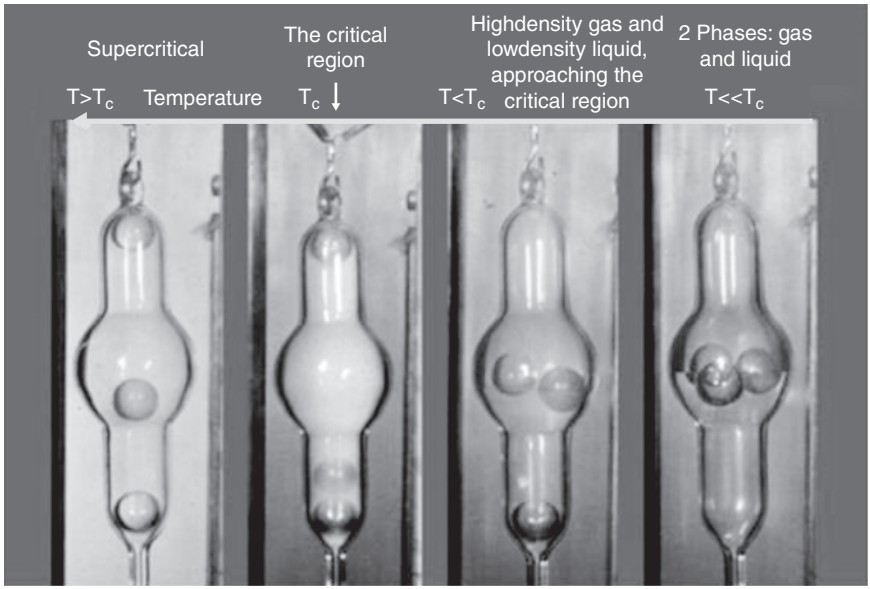

Figure 1.6 Behavior of four spheres of different densities in CO_2: supercritical, critical, subcritical, and liquid (0.92 g/mL). *Source:* Chemical & Engineering News, June 10, 1968, p. 105, Photo by Ray Rakow.

the liquid phase, which indicated that the liquid phase density had increased even more allowing even the greatest dense sphere to float on its surface.

During the late 1890s, numerous studies of high-pressure fluids and solubilization phenomena were recorded. For example, Amagat in 1879 performed high-pressure experiments using mercury columns that extended to the bottom of mine shafts [17]. Later, Cailletet (1891) used a mercury column from the top of the Eiffel Tower for high-pressure experiments [18]. By changing the density of the fluid through temperature and pressure variation, the solvation strength of a supercritical fluid was altered. An increase of the pressure caused the density of the supercritical fluid to increase thereby causing it to become more liquid-like. When the temperature was increased, the density of the supercritical fluid decreased, and the phase became more gas-like. Depending upon the density, the viscosities of supercritical fluids were thought to be similar to gases or intermediate between gases and liquids.

1.3 Supercritical Fluid Chromatography (1962–1980)

Considerable time passed before the previously described basic knowledge regarding supercritical fluids was utilized for SFC. It was first proposed in 1958 by James Lovelock while at Yale University [19]. He conceived the idea of using

supercritical water, ammonia, sulfur dioxide, and carbon dioxide for chromatographic mobile phases in open tubular columns to increase the solvating power of the mobile phase in order to elute nonvolatile ionic substances, but he surprisingly did not attempt to practice the art of SFC. He, nevertheless, related his ideas concerning SFC to his coworkers Sandy Lipsky and Ray Landowne, who urged him to commit his thoughts and findings to paper. Lovelock, however, realized that the high liquid-like density, high gas-like diffusivity and low viscosity of fluids above the critical point would extend both GC and LC. Based upon these early discussions, he was reported to have had his research document notarized and witnessed soon after, but no patents were ever noted. He also suggested the name "critical state chromatography" for the separation. It was noted that acting as a solvent is a critical characteristic that differentiates the compressible mobile phases used in SFC from gas chromatography (GC).

Supercritical fluid chromatography was invented by gas chromatographers who initially explored gas at high pressures. Their hope was to elute compounds that could not be analyzed with GC because the analytes were prone to decompose at the temperatures needed to elute them. Ernst Klesper in 1962 employed a 30-inch long column packed with 33% Carbowax 20M on Chromosorb W (diatomaceous earth, GC packing, 180–250 µm), and is considered to be the first person to use higher gas pressures to elute, for example, porphyrin mixtures at lower temperatures than required for their elution using traditional GC conditions. Supercritical temperatures were maintained to enable the gas pressure to be continuously increased without it passing through vapor–liquid biphasic conditions [20]. In 1967, Sie and Rijnders were the first to use the term "supercritical fluid chromatography" for this new chromatographic technique. They also suggested the use of mobile phase pressure programming.

Later, Jentoft and Gouw were the first to employ mobile phase modifiers (i.e. methanol in n-pentane) to control retention in SFC. Figure 1.7 shows the pressure–density relationship for CO_2 in terms of reduced parameters (e.g. pressure, temperature, or density divided by the appropriate critical parameter) including the two-phase vapor–liquid region used in these earlier studies. This relationship is generally valid for most single-component systems. The isotherms at several reduced temperatures show the variation in density that can be expected with changes in pressure. Thus, the density of a supercritical fluid will be typically 100–1000 times greater than that of a gas at ambient temperatures. Consequently, molecular interactions increase due to shorter intermolecular distances.

The temperature ranges in supercritical fluid processes depend on the fluid used and reflect the respective critical values. The majority of supercritical work is done with CO_2, which has a T_c of 31.30°C (304.6 K). Critical properties of select compounds of differing polarity are shown in Table 1.3. The physical properties of CO_2 and other supercritical fluids are also summarized in the table.

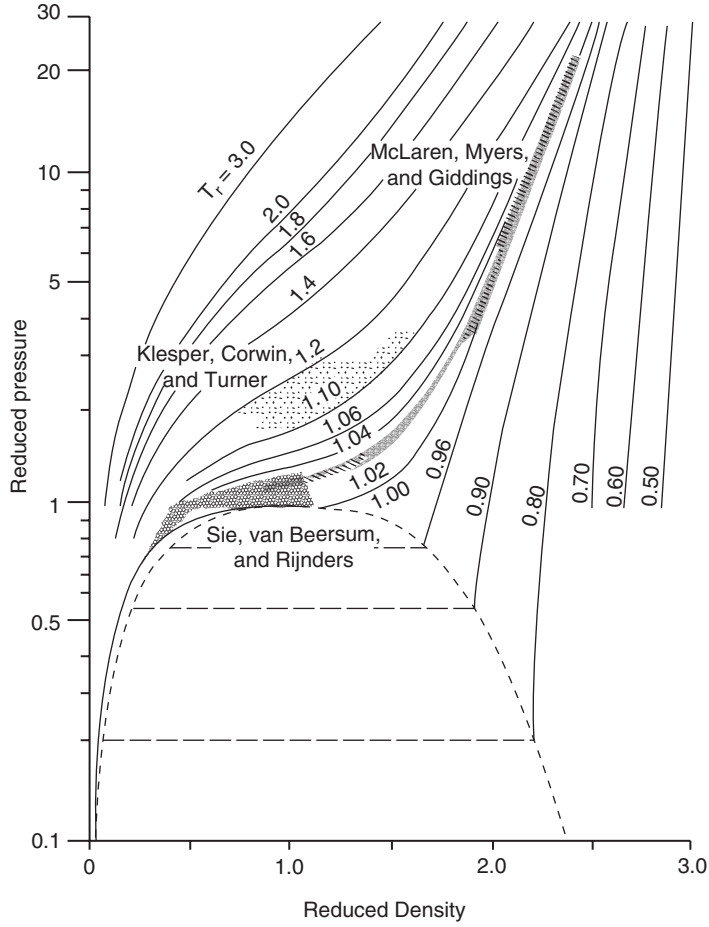

Figure 1.7 CO_2 pressure as a function of density at different temperatures expressed in terms of reduced parameters. The area below the dotted line represents the two-phase gas/liquid equilibrium region. The indicated areas refer to conditions at which the researchers performed their experiments during the initial studies of SFC. *Source:* Reproduced with permission of Milton L. Lee.

Klesper was born in Cologne, Germany and later finished his doctorate in inorganic chemistry in 1954 at the University of Hamburg. From there he moved to the United States, took an industrial position with the William T. Burnett Company in Baltimore, MD as a chief chemist. Thereafter he became a Research Associate at the Johns Hopkins University and an Assistant Professor at the University of Maryland. Subsequently he became associated with the Institut fur Macromolekulare Chemie at the University of Freiburg where he came into contact with polymer chemistry. He then joined A.H. Corwin's laboratory at Johns Hopkins University in Baltimore where the first experiments using

Table 1.3 Physical parameters of selected supercritical fluids.

Fluid	Dipole moment (debyes)[a]	T_c (°C)[a]	P_c (atm)[a]	ρ_c (g ml^{-1})[a]	ρ_{400} (g ml^{-1})[b]	ρ_1 (g ml^{-1})[a,c]
CO_2	0.00	31.3	72.9	0.47	0.96	0.71 (63.4 atm)
N_2O	0.17	36.5	72.5	0.45	0.94	0.91 (0 °C) 0.64 (59 atm)
NH_3	1.47	132.5	112.5	0.24	0.40	0.68 (–33.7 °C) 0.60 (10.5 atm)
n-C_5	0.00	196.6	33.3	0.23	0.51	0.75 (1 atm)
n-C_4	0.00	152.0	37.5	0.23	0.50	0.58 (20 °C) 0.57 (2.6 atm)
SF6	0.00	45.5	37.1	0.74	1.61	1.91 (–50 °C)
Xe	0.00	16.6	58.4	1.10	2.30	3.08 (111.75 °C)
$CC1_2F_2$	0.17	111.8	40.7	0.56	1.12	1.53 (–45.6 °C) 1.30 (6.7 atm)
CHF_3	1.62	25.9	46.9	0.52	1.15	1.51 (–100 °C)

[a] Data taken from references [21, 22].
[b] The density at 400 atm and T_r = 1.03 was calculated from compressibility data [23].
[c] Measurements were made under saturated conditions if no pressure is specified or were performed at 25 °C if no temperature is specified.

supercritical fluids as the mobile phase were performed in collaboration with Corwin and Turner as far back as 1962, which was interestingly well before the introduction of high performance liquid chromatograph (HPLC).

In Klesper's first reduction to practice a mixture of nickel porphyrins (etioporphyrin II, nickel etioporphyrin II, and nickel mesoporphyrin IX dimethylester) was separated with dichlorodifluromethane and monochlorodifluoromethane at pressures above 1000 and 1400 psia, respectively, as the mobile phase [20]. These particular mobile phases were chosen because of their low flammability and physiological inertness. The instrumentation was very simple but well suited to demonstrate the potential of employing supercritical fluids as the mobile phase in chromatography. At the time numerous supercritical fluid separations were presented and reported in the literature which were thought to be superior to existing gas and liquid chromatography reports. SFC was eagerly thought to be a future replacement for GC and LC of polar and high molecular weight components. See Table 1.4.

A diagram of the apparatus is shown in Figure 1.8. No mechanical pump or elaborate detector was used. The column was contained in a glass, high pressure gauge tube which allowed observation of the colored bands of porphyrins which moved down the column. The method was at that time called "high pressure gas chromatography" instead of SFC. The first publication by Klesper's group was a

Table 1.4 Early attraction of supercritical fluid chromatography (SFC).

• Capable of generating GC peak efficiencies
• Separations operated at higher flow rates than LC

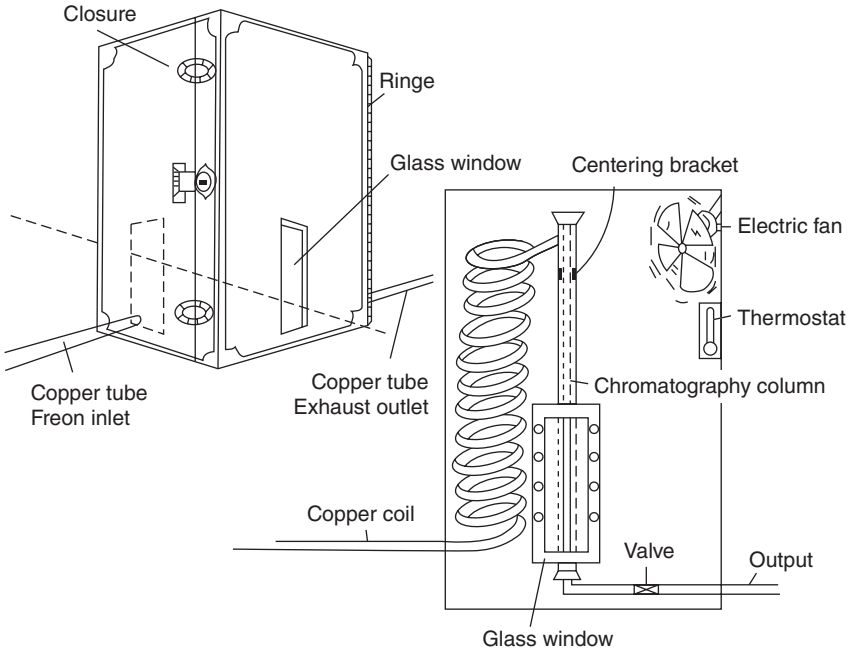

Figure 1.8 Diagram of apparatus used by Klesper to demonstrate proof of concept for first SFC. *Source:* Reproduced with permission of Milton L. Lee.

"Communications to the Editor" in the Journal of Organic Chemistry entitled "High Pressure Gas Chromatography Above Critical Temperatures [20]."

Klesper continued his research on polymers and SFC at the University of Freiburg in Germany [24]. The influence of temperature, pressure, and flow rate on the behavior of dimethylether and diethylether plus the separation of oligomers with UV-absorbing side groups via SFC using eluent gradients [25], and the elution behavior of styrene oligomer fractions in SFC [26] were just three of the early studies conducted by Klesper. By this time, Ernst Klesper was justifiably known in separation science as the "Father of Supercritical Fluid Chromatography" based upon his outstanding publication record in the field [27].

In 1968, Giddings et al. published an article concerning the influence of high pressures on retention in GC with carbon dioxide and ammonia as the mobile phase [28]. He stated that the use of high pressure in chromatography

"would cause the convergence of gas chromatography with classical liquid chromatography." A decade later, Giddings also studied the general aspects of pressure-induced equilibrium shifts in size exclusion chromatography [29]. However, this new form of chromatography was generally noted as "dense gas" or "high pressure gas chromatography" rather than SFC.

Before Klesper and Giddings, other chromatographers were studying the use of high pressure gases and supercritical fluid mobile phases. The work of Riki Kobayshi at Rice University can be cited here. Kobayashi was also investigating high pressure equilibria by chromatography, but he was not using the increased solvent power of the media to elute nonvolatile materials. It was Sie, VanBeersum, and Rijnders in 1966 at the Shell Laboratories in Amsterdam, who first published the separation of C_7 to C_{13} n-paraffins, and soon after "supercritical fluid chromatography" was coined by the same workers in 1966–1967 [30]. Pressure programming and the use of mobile phase modifiers (i.e. methanol in n-pentane) were later (1969–1970) also demonstrated by Gouw and Jentoft to control retention [31, 32]. The prolific Amsterdam group published numerous papers during this time such as (a) the effect of mobile phase velocity and particle size on column plate height, (b) the use of porous polymeric stationary phases, and (c) numerous early useful reviews on SFC [33, 34], Table 1.5.

After the initial period of great interest in packed column supercritical fluid chromatography (pcSFC) in the 1960s, the progress of SFC slowed. In part, the slow development was due to several factors, such as (a) early experimental problems, (b) the lack of commercially available instrumentation, and (c) the fact that SFC development was over-shadowed by simultaneous developments in HPLC. A more striking reason surfaced in the late 1960s when several workers in the area suggested that the efficiency of crudely packed columns seemed to degrade with higher column pressure drops. Gouw and Jentoft postulated that decreasing density along the column axis acted like a decreasing temperature gradient in GC, causing a loss in efficiency [34]. It was, therefore, claimed from selected studies by various separation scientists that SFC packed columns could never produce more than ~20 000 theoretical plates, and at the time particles smaller than 5 µm were virtually ruled out in this research. Later it

Table 1.5 Milestones in the development of SFC.

(1996) First publication: C_7–C_{13} n-paraffins
(1967) Coined supercritical fluid chromatography
(1970) Convergence of high pressure GC and LC
(1970) Pressure programming and modifiers
(1974) Negative temperature programming
(1981) Open tubular column SFC
(1983) Density programming
(1984) Practical SFC with flame ionization detection

was learned that many of these problems stemmed directly from (i) inadequate use of home-made equipment related to back pressure control and (ii) the use of fixed restrictors instead of more effective back pressure regulation and variable restriction. Van Wasen et al. identified yet another reason around this time for the slow development of SFC [35]. They proposed that progress in SFC development was severely obstructed by the lack of physico-chemical knowledge regarding supercritical fluids. Unfortunately, this became a perception that existed for another 20 years.

The term "pressure drop" in chromatography refers to the decrease in pressure as the eluent is pumped through the column. The primary source of the system back pressure is the column, and it is proportional to the viscosity of the mobile phase. The magnitude of the pressure drop for the different types of chromatography increases in the order GC < SFC < HPLC. However, the effect of the pressure drop on the separation efficiency of the column is greatest in SFC because changes in pressure do not affect to any great extent the solvating abilities of liquids and gases. On the contrary, in SFC, the solvent properties of the supercritical fluid are greatly affected by the drop in pressure along the column. As the pressure of the supercritical fluid decreases, the density and consequently the solvent strength of the supercritical fluid decreases. As a result of the lower solvent strength, the peaks broaden instead of becoming narrower, and the total efficiency of the system decreases [36].

SFC in the 1970s experienced another dormant state. Research interest in SFC was limited; although some very important work was published by several groups during this period. For example, Schneider and Bartmann continued their research on the physicochemical aspects of supercritical fluids with studies on the density dependence of (a) retention factors, (b) binary diffusion coefficients, and (c) partial molar volumes [37]. The same co-workers also introduced SFC with negative temperature programming. More practical SFC investigations were introduced by Rogers and Graham regarding the separation of oligomers by a combination of pressure, temperature, and mobile phase programming [38]. Options afforded by supercritical fluids, nevertheless were beginning to be readily recognized (Table 1.6).

Table 1.6 Options afforded by supercritical fluids.

- Adjustable solvating power
- Greater and more variable diffusivity than liquid
- Lower and more variable viscosity than liquid
- Negligible surface tension
- Low temperature efficient separations
- Nontoxic mobile phase
- Analyte isolation achieved by pressure reduction

Table 1.7 Advantages: SFC vs. GC.

• Lower temperature
• Much higher molecular weight range
• Large programming range
• Selectivity tuning via pressure control
• Selectivity tuning via temperature control
• Universal mass detection via flame ionization

Hybrid chromatographic techniques made their first appearance in the late seventies as SFC was interfaced to mass spectrometry by Randall and Wahrhaftig [39]. In 1971, Novotny et al. published an important paper concerning the effects of temperature, pressure, eluent composition, flow rate, and type of stationary phase on retention factors [40]. It should be noted here that during the time period between 1962 and 1980, nearly all of the publications that related to SFC (which totaled about 90) involved the use of packed stainless steel columns. With the advent of capillary columns in 1980, SFC experiments were about to drastically change. Advantages of SFC versus GC (Table 1.7) were becoming very popular.

1.4 SFC with Open Tubular Columns (1980–1992)

A strong revival of interest in SFC occurred in the early eighties for two reasons. One important aspect was the introduction in 1981 of a commercial kit that converted a Hewlett-Packard model 1084 HPLC into an SFC system [41]. The resulting instrument, which was greatly due to the combined efforts of Berger, Gere, Lauer, and McManigill, was again for packed column SFC [42, 43]. A striking feature of this new instrumentation was that flow rate, mobile phase composition, column temperature, and column outlet pressure were all independently controlled. Unfortunately, there was no pressure programming and detection was exclusively UV.

The modified instrument, however, permitted operation with liquid CO_2 at the pump stage while maintaining supercritical conditions at the separation stage. Stationary phases were mostly standard, spherical silica bonded phase columns borrowed from HPLC. When the SFC-*incompatible* HP model 1090 HPLC system was introduced to the public, the HP 1084 model for SFC was withdrawn from the market, thus ending production of the **first** commercial SFC system that shocked the previously growing SFC market [43]. To partly fill the gap, it should be noted that in 1985, JASCO introduced a combined supercritical fluid extraction/supercritical fluid chromatography system which was similar to the modified but outdated HP 1084 system. The JASCO

featured, however, the first electronic back pressure regulator specifically designed for SFC. User interest in this instrumentation, however, never lived up to expectations [44].

The introduction of open tubular wall coated columns in SFC by Lee and Novotny using pressure programming of pure CO_2 was reported in 1981 [45]. This development came at about the same time that Berger's group at Hewlett Packard reported the invention of fused-silica capillary columns which were capable of withstanding the high pressures necessary for open tubular column SFC (otSFC) [41]. otSFC experienced an explosive growth wherein the number of publications increased dramatically [46] because it was deemed by workers in the field to be more applicable (after considerable heated debate) for otSFC than for pcSFC. As a result, the otSFC technology was commercialized in 1986 through Lee Scientific (Salt Lake City, UT), which was later acquired by Dionex Corporation. The development of pcSFC was thus stymied and subsequently put on hold again as capillary columns dominated the development of both theory and instrumentation in SFC for the next five to seven years [47, 48] even though the earlier popular opinion regarding open tubular columns would be shown to be without merit.

The first workshop totally devoted to users of SFC was conducted in January 11–14, 1988 at Park City, UT. The meeting was sponsored by the State of Utah and Brigham Young University. The program attracted 175 participants from all over the world. The workshop schedule was demanding (8:00 a.m.–10:00 p.m.) with 39 presentations of 20 minute duration. Each session was terminated by an hour long discussion of a particular aspect of SFC. At the beginning of the conference, Professor Ernst Klesper was presented a plaque for his pioneering work in SFC. His plenary lecture considered various gradient methods (temperature, pressure/density, and eluent composition) in SFC and their overall effect on resolution. The workshop closed on a high note of optimism regarding the potential of SFC, but everyone agreed that much research and developmental work needed to be performed.

In the early days, otSFC instruments resembled GC instruments, but the former used a syringe pump as a pressure source to change the CO_2 density and solvating power of the mobile phase. A homemade tapered fused silica fixed restrictor to limit flow through the small diameter chromatography column was employed. Detection was primarily via high temperature flame ionization. The simplicity of such an approach was for a time overwhelming such that most accompanying research activity replaced pcSFC usage. At one point, there were six to seven companies manufacturing and selling capillary SFC's and only one (JASCO) selling packed SFC columns [43].

Some of the immediate benefits of otSFC included (i) lower operational temperatures than GC, (ii) much higher molecular weight range than GC, (iii) large programming range primarily via pressure adjustment, (iv) selectivity tuning via temperature change, (v) universal mass spectrometric detection

coupled with flame ionization detection, and (vi) amenable to very polar and some ionic analytes via pre-column derivatization [49, 50]. Figure 1.9 illustrates the separation of a polymeric material with three open tubular columns each with a different bonded phase. Pressure programming was employed in each case, yet separations were time consuming. Open columns possessed a high efficiency if operated under optimum conditions. In the Figure, separation in each case appears to be by oligomer unit. The time required to generate a greater plate number under this situation largely depended on both the inner

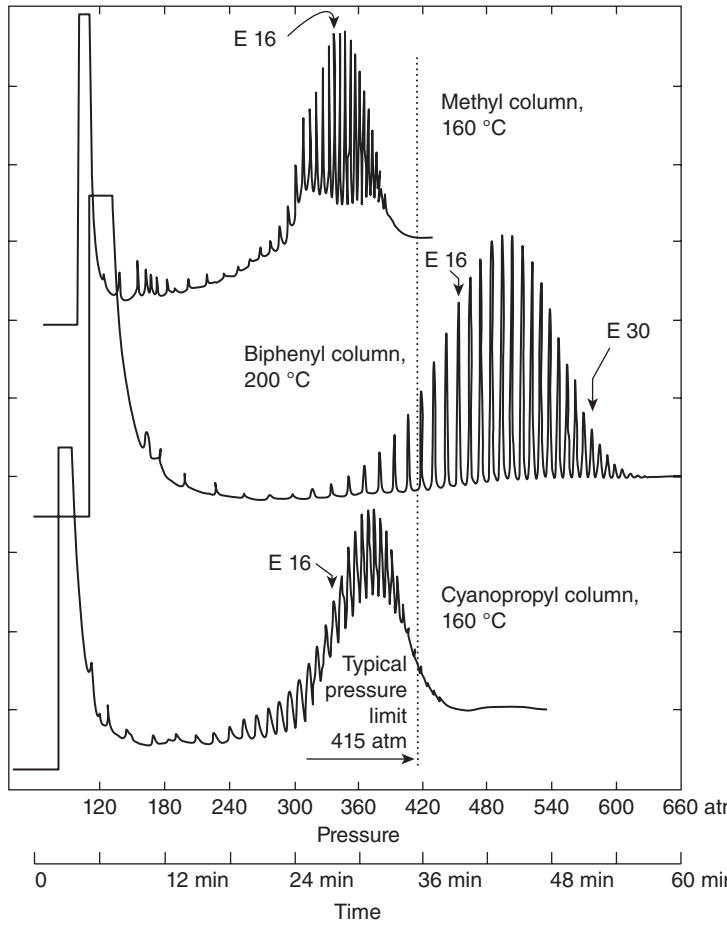

Figure 1.9 Separation of a polymeric material with three open tubular columns each with a different wall coated stationary phase. Pressure programming was employed in each case; yet separations were time-consuming. Separation in each case appears to be by oligomer unit. *Source:* Reproduced with permission of Wiley. Chester [52].

diameter of the column and the diffusivity of the analyte in the mobile phase which apparently was not the same as evidenced by the varying retention time of the E-16 oligomer. The slower the diffusion in the mobile phase, the smaller the inner diameter of the open tubular column had to be in order to obtain a given plate number. As diffusion in supercritical fluids is much slower than in gases, the inner diameter of open columns in otSFC was always much smaller than in GC. Consequently, a SFC column diameter of 50 µm was typically used.

The use of narrow open columns, however, imposed severe requirements on the instrumentation [47]. Sample injection, post column pressure/flow restrictors, and analyte detection devices were notable points of study. Flow split, timed split, and combined split injection techniques allowed the introduction of nanoliter sample sizes onto open columns with inner diameters below 50 µm. Total injection without splitting, on the other hand, was achieved by the use of a retention gap combined with a solvent venting exit. A number of fixed restrictor designs were also developed to control flow and pressure such as the polished integral tapered restrictor and the frit restrictor. The sensitivity of the detection devices required special consideration due to the reduced sample capacity of narrow-bore open columns. Furthermore, stationary phases in contact with supercritical fluids (i.e. otSFC) had to be highly cross-linked in order to impede column bleed. Thus, the employment of GC open tubular columns for otSFC where the mobile phase was supercritical CO_2 was not chromatographically successful. This feature dictated the use of columns that exhibited the best levels of deactivation attainable.

otSFC technology, in spite of the instrumental challenges, flourished for some time in both academic and industrial laboratories, but it had several disadvantages for wide-scale acceptance by the analytical community [46, 50]. Specifically, the technique, which relied mostly on nonpolar stationary phases, was traditionally limited to relatively nonpolar analytes because (i) pure CO_2 as the mobile phase exhibited poor solvating power similar to hexane, (ii) robust sample injection was not feasible, (iii) a wide polarity range of stationary phases was not available, (iv) high quality separations required ~60 minutes, (v) gradient CO_2 pressure and flow rate were coupled such that flow rate changed as mobile phase pressure changed, (vi) passive, fragile fused silica restrictors at the column outlet often plugged causing the separation to be aborted, and (vii) multi-gram scale-up of a successful separation was not feasible. Unfortunately, the technique was oversold as being appropriate for more polar analytes.

Still, in the 1980s, pcSFC was not very popular in spite of the fact that packed columns were thought by some separation scientists to be generally much easier to operate than open tubular narrow columns. Furthermore, columns packed with sub-10 µm particles were demonstrated to be more time efficient than contemporary, open columns. A fundamental problem, however, of packed columns in SFC continued to be the inherently high column pressure

Table 1.8 SFC was in a bad shape in 1980s.

• Fragile fixed restrictors/no flow control
• Pre-mixed cylinders of modified CO_2
• Helium-padded CO_2
• High temperatures/Long retention times
• Open tubular columns in/packed columns out
• No long-term vendor commitment
• Lacked HPLC figures of merit/Extension of GC
• Amenable only to nonpolar analytes

drop [51]. As pressure is an extremely important parameter in SFC, a marked change in the properties of the mobile phase along the column was anticipated to occur in the presence of a significant pressure drop. In contrast, the pressure drop across the column was negligible for otSFC.

Later in the decade, consensus was reached that both column types have their own unique advantages and disadvantages. History has, however, revealed that more highly significant technological developments have occurred in pcSFC than in otSFC, which accounts for the higher popularity and applicability for problem solving of the former than the latter. In the early days, capillary SFC and packed column SFC co-evolved and competed with each other. Capillary SFC lost ground and eventually faded away, mainly as a result of poor reproducibility issues. The early systems were costly and not user friendly, resulting in the technique being marginalized as too expensive and inefficient. The various features of SFC in the 1980s are listed in Table 1.8.

1.5 Rediscovery of pcSFC (1992–2005)

pcSFC underwent another renaissance in interest near the beginning of the 1990s when (i) the long-term limitations of otSFC became obvious and (ii) important progress in pressure gradient techniques involving mixed mobile phases was achieved for pcSFC. Today, it is generally conceded that dramatic differences exist between the instrumentation required for use of packed columns and open tubular columns in SFC. In fact, the use of open tubular instrumentation with packed columns often results in complete failure. otSFC resembles GC at high pressures, except that pressure (or density) programming in open tubular SFC has replaced temperature programming in GC. Because otSFC is still controlled using a passive fixed restrictor, flow varies with pressure, temperature, and mobile phase composition. Furthermore, the flow rate is typically measured in μL/min, and solutes are usually quantified with a flame ionization detector after the mobile phase has expanded to

atmospheric pressure. In retrospect, otSFC has been demonstrated with a host of flame based detectors such as electron capture and nitrogen chemiluminescence. Few papers nowadays are published that deal with otSFC, and vendor support for otSFC technology has practically disappeared.

pcSFC with enhanced sophisticated instrumentation that allowed independent flow control under pressure gradient conditions became available in 1992. pcSFC was generally much easier to operate because it resembled HPLC wherein binary and ternary mobile phases were common. Then, it was realized that the composition of the mobile phase was almost always more important than mobile phase density programming in controlling retention, akin to HPLC in this regard. Flow rates were typically several mL/min. Pressure was controlled by an electronically controlled back pressure regulator (BPR) mounted at the end of the column. As previously noted, a fundamental problem of pcSFC during this period continued to be the inherently high column pressure drop [51]. As pressure was an extremely important parameter in pcSFC, a marked change in the properties of the compressible mobile phase along the column was predicted to occur in the presence of a significant pressure drop. Standard ultra-violet detectors with a high-pressure flow cell pre-BPR or a mass spectrometer post-BPR were used for detection and quantification.

Interest in pcSFC during this period was higher than ever because pcSFC was capable of generating peak efficiencies similar to those observed in GC and separations could be run at much higher flow rates than in HPLC. Furthermore, pcSFC was scalable, detector friendly, and economical. It afforded both rapid method development and a more environmentally acceptable chromatographic process than previously encountered with SFC [53]. pcSFC usually used (i) silica-bonded stationary phases, (ii) binary or ternary fluids, (iii) composition programming, and (iv) an ultraviolet detector.

Once modifiers were added to the CO_2 mobile phase, composition became even more important than CO_2 pressure or density in determining retention, unlike the situation that prevailed with otSFC. Packed columns are usually operated near the critical temperature of the fluid with flow control pumps and electronically controlled back pressure regulators mounted downstream of the column to obtain accurate flow rates and mobile phase composition. The combination of upstream flow control and downstream pressure control allowed both volumetric mixing of the main fluid and gradient modifier elution. pcSFC became viewed as an extension or subset of HPLC. Unfortunately, when one attempted to use inappropriate open tubular instrumentation with packed columns, the results were usually complete failure; whereas the use of packed column instrumentation with open tubular column instrumentation soon was realized to be entirely feasible.

With the availability of more reliable pcSFC instrumentation, a ready solution to several major analytical problems became economically and environmentally feasible. For example, pcSFC affords many advantages in comparison to

HPLC not the least of which is the rapid *separation of enantiomers* because chiral selectivity usually increases with decreasing temperature. Low temperature also reduces the risk of analyte racemization and thermal decomposition. Furthermore, CO_2 replaces petrochemically derived hydrocarbons that are used in the mobile phases of HPLC separation, resulting in a reduction in solvent utilization by as much as 90%. To successfully perform chiral pcSFC, a polar modifier must be added to the CO_2 mobile phase and in many cases operation across a gradient may exist during the separation.

pcSFC is ideal for preliminary rapid chiral screening. A fast chiral screen is used for rapid discernment of the most appropriate stationary phase and modifier. Finding a highly selective chiral stationary phase is a very key step in method development of a chiral separation. In addition, modifiers and additives usually play a significant role in enantiomeric recognition. A number of screening strategies have been reported that take advantage of short columns, high-flow rates, and fast gradients.

Preparative separations received considerable attention during the rediscovery of pcSFC [53–56]. The decrease in solvent use and waste generation when using CO_2 mobile phases compared to conventional liquid phases makes preparative SFC especially attractive for providing purified materials on a kilogram scale. Furthermore, the product is recovered in a more concentrated form relative to HPLC, thereby greatly reducing the amount of solvent that must be evaporated. The higher pcSFC flow rates also contribute to higher productivity relative to HPLC methods. The faster pcSFC process makes the separation cycle time significantly shorter such that it is viable to make purification runs by "stacking" small injections in short time windows without compromising the throughput. In this way, the utilization rate of expensive column material is much higher than with conventional preparative columns.

The unique properties of supercritical fluids have had a broad impact across the wider field of separation science through the development of so-called "unified chromatography" whose underlying principle was that there are no theoretical boundaries between mobile phases. Roger Smith pointed out in 1999 [48] that probably the most important idea that supercritical fluids have brought to separation science is a recognition that there is unity in the separation method and that a continuum exist from gases to liquids. Tom Chester noted during this period that when viewed from the perspective of the mobile phase, the perceived complexities of old and new modes of chromatography are not so complex after all [57]. Calvin Giddings also called attention to this notion over half a century ago and made the observation that as the column diameter decreases so do the differences between chromatographic techniques [28, 29]. As has hopefully been evident thus far in this chapter, knowledge of the history of SFC truly brought separation methods together near the close of the twenty-first century.

1.6 Modern Packed Column SFC

In the 1990s and in the early 2000s, pcSFC struggled to establish itself in other applications beyond preparative scale chiral separations. At the time, the technique's repeatability and robustness were still below those achieved in HPLC, and thus these issues hindered the implementation of SFC in both analytical and QC laboratories. SFC is experimentally different than LC, mainly because of the compressibility of the mobile phase. In this regard, Fornstedt has stated that SFC can be thought of as a "rubber variant" of HPLC where everything considered constant in HPLC varies in SFC [58]. The good news is that reluctance of analysts to use pcSFC has faded since the introduction by several major vendors of a new generation of SFC instruments dedicated to analytical purposes by several important manufacturers has happened. These new systems (first bullet point in Table 1.9) have benefitted from a novel automated back pressure regulator design and from ultra-high performance liquid chromatography (UHPLC) technology, which incorporates higher pressure limits and reduced void volumes. Improved performance, reliability, and full compatibility with most modern stationary phases (sub-3 μm core shell and fully porous sub-2 μm particles) have greatly broadened the application spectrum of this technique, making modern pcSFC with CO_2-based mobile phases competitive and complementary to UHPLC [58].

In modern pcSFC, the control of density is crucial. If not properly managed, the solvent strength differs between analyses leading to shifting retention times. The automated back pressure regulator (ABPR) is responsible for the pressure control, so proper design is of crucial importance. Changes in the temperature of the incoming CO_2 can also result in shifting retention times. In addition to these issues, the injection volume flexibility was limited in older generations of pcSFC instrumentation reflecting imperfections in the design of the partial loop injector for SFC. From a detection perspective, the UV noise level caused by differences in the refractive indices of mobile phase constituents needed to be minimized. Thus, the revival of interest in pcSFC in recent years has been predominantly the availability of state of the art instrumentation [58]. The ACQUITY system was commercially introduced in 2012 by Waters Corp. (Milford, MA, USA). To stress its differences from previous generations of pcSFC equipment, the term "convergence chromatography,"

Table 1.9 Reproducible flow and gradient delivery were keys to the re-emergence of SFC.

- Constant flow pump/variable restrictor. Linear velocity decreased with density increase. Efficiency remained constant
- Pressure controlled pump/fixed restrictor, mass flow increased with pressure increase. Efficiency decreased

originating from a statement of Giddings, was introduced by Waters [59]. Important features included the efficient cooling of the CO_2 pump heads by Peltier cooling and the design of a new dual stage ABPR that was heated to avoid frost formation. The maximum flow rate and pressure of the instrument was 4 mL/min and 413 bar, respectively. The column outlet was connected to a photo-diode array detector, which included a high pressure UV cell with a volume of 8 µL and a path length of 10 mm. The instrument could be controlled by typical software packages such as Empower or MassLynx.

The 1260 Infinity SFC/HPLC system was introduced by Agilent Technologies (Waldbronn, Germany), also in 2012 like the Waters system. The Agilent system is a hybrid that allows both UHPLC and pcSFC separations [60, 61]. Using switching valves and two pumps (one for SFC – Pump A and one for UHPLC – Pump B), the system can be modified based upon the needs of the user. Earlier collaboration (2010) between Aurora and Agilent Technologies resulted in the introduction of a dedicated analytical SFC module (1260 Infinity Analytical SFC system). The module is responsible for the compression of the incoming gaseous CO_2 with temperature control via a chilling liquid. The pre-compression requirement is removed from the other pump, which now functions only as a metering pump to control the flow of liquid CO_2. The BPR in this apparatus is a heated single stage device. Compared to traditional pcSFC systems, which employ a single pump to both compress the liquid CO_2 and meter the required flow, this approach has been shown to significantly reduce baseline noise. The maximum flow rates and pressures are 5 mL/min and 600 bar, respectively. The photodiode array is equipped with a micro flow cell with a volume of 1.7 µL and a 6 mm path length that is resistant to a pressure of 400 bar. The 1260 Infinity Analytical SFC system is controlled via ChemStation or OpenLab [62, 63].

One's view of SFC today is entirely different from that of 25 years ago when flow rates and gradient delivery were not reproducible, analytical UV sensitivity was not acceptable, and stationary phases were designed for reversed phase chromatography as opposed to normal phase. Today, SFC is considered to be primarily normal phase using mostly the same hardware and software developed for HPLC. The mobile phase is a binary or ternary mixture with CO_2 as the main component. The separation is usually performed as a gradient elution where the composition of the mobile phase is changed versus time. Polar stationary phases such as bare silica and 3-aminopropylsilica are routinely employed. pcSFC has numerous practical advantages relative to reversed phase HPLC such as higher speed throughput, more samples per day, more rapid equilibration, and shorter cycle time. SFC yields lower operating cost and lower column pressure drop, and it is orthogonal to reversed phase HPLC. Solvent consumption is low; therefore, waste generation is minimal. Finally, compounds of interest can be isolated in a relatively small amount of solvent because CO_2 vaporizes away.

The reluctance of analysts to use pcSFC has faded since the recent introduction of a new generation of instruments dedicated to analytical purposes by several important manufacturers [64]. These new systems benefit from a novel BPR design and are largely based on UHPLC technology. Improved performance, reliability, and full compatibility with the most modern stationary phases have greatly broadened the application spectrum of this technique. This text will draw heavily upon this advanced technology as it deals with a variety of analytical and preparative "real world" applications. Pedagogy as it relates to methods development involving a host of polar, nonpolar, and bipolar analytes will be emphasized. Applications to polymeric materials, natural products, polar water soluble analytes, surfactants, organic salts, fatty acids, lipids, organometallics, etc. are experiencing great success. Depending upon the nature of the stationary phase, reversed phase pcSFC, ion pairing pcSFC and aqueous promoted HILIC pcSFC can be expected to augment the more popular normal phase pcSFC that has historically been known for decades.

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