Modern Supercritical Fluid Chromatography

Carbon Dioxide Containing Mobile Phases

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Preface

0.1 Scope of the Book

Supercritical fluid chromatography (SFC) is more than 50 years old. Chapter 1 entitled "Historical Development of SFC" recaps over a much greater timeframe of the discovery of supercritical fluids and their development as a medium for chromatographic separation of both volatile and nonvolatile analytes. A real interest in SFC using either packed or open tubular columns began in the early 1980s when the first commercial preparative SFC instrument became available [1]. This development led to growing interest in the separation of stereoisomers which started with the pioneering work of Frenchman Marcel Caude and his research group in 1985 [2]. Thus, a wide variety of chiral separations were reported and applied near the turn of the century employing both analytical and preparative packed column (pcSFC) technology. SFC with open tubular columns (otSFC) also peaked in the 1980s but fizzled during the following decade. Interest in pcSFC is currently higher than ever before. For example, the technique is capable of generating peak efficiencies approaching those observed in gas chromatography (GC). On the other hand, pcSFC separations can achieve much higher efficiencies per unit time than in high performance liquid chromatography (HPLC). pcSFC has embraced a critical mass of separation scientists and technicians in terms of the number of workers in the field worldwide. Hundreds of supercritical fluid chromatographs currently are in use. Furthermore, pcSFC is (i) detector and environmentally friendly,

(ii) interfaceable with sample preparation, (iii) relatively economical in cost, and (iv) is a superior purification tool. Chapters 3 and 4 provide discussion of these critical developments that earlier had been referred to as *dense gas chromatography* [3]. Related work in the field currently uses both supercritical and subcritical mobile phase conditions to perform separations as well as purifications.

During the past 20 years, pcSFC has created a *bonafide* niche for itself as the go-to workhorse in chiral separations. Chapter 6 discusses in detail this topic. It has afforded many advantages for rapid separation of enantiomers over HPLC due to its greater separation efficiency per unit time. The advantages of pcSFC over HPLC which are also discussed later in the book, however, are practical but not fundamental. The greatest difference between pcSFC and pcHPLC is just simply the need to hold the outlet pressure above ambient in separations in order to prevent expansion (i.e. boiling) of the mobile phase fluid.

Enantiomeric separations are more compatible with ambient SFC than with high temperature HPLC because chiral selectivity usually favors decreasing temperature wherein the risk of analyte racemization is minimized. On the other hand, the risk of analyte thermal decomposition as in the GC of cannabis – related components is lessened. Furthermore, the straightforward search (primarily by trial and error) for a highly selective chiral stationary phase is a key step in the development of chiral pcSFC separations that address *industrial* applications. In this regard, a number of screening strategies that incorporate a wealth of stationary phases are discussed in the book that take advantage of short columns, small particles, high flow rates, and fast gradients.

Upon scale-up of analytical chromatography to preparative supercritical fluid separations as discussed in Chapter 8, the resulting decrease in solvent usage and waste generation relative to preparative scale HPLC is strikingly dramatic. SFC product can be routinely recovered at higher concentration relative to HPLC which greatly reduces the amount of mobile phase that must be evaporated during product isolation. Higher SFC flow rates contribute to higher productivity. The faster SFC process makes the separation cycle time significantly shorter such that it becomes practical as well as feasible to make purification runs by "stacking" small injections in short time windows without compromising throughput. Table 0.1 lists additional advantages of supercritical fluid chromatography.

Table 0.1 Advantages of supercritical fluid chromatography.

- High diffusivity/low viscosity yield greater resolution per unit time.
- Longer packed columns afford greater number of theoretical plates
- Low temperature reduces risk of analyte isomerization
- Scale-up of separation and isolation of fractions are facilitated

pcSFC (as most analytical techniques) has had a tortuous development history, but it appears that analytical and preparative scale chiral SFC are currently on the firmest foundation ever experienced with vendors that are strongly committed to advancing the technology. Extensive, new developments in achiral SFC and a much broader spectrum of applications outside the pharmaceutical area are already happening. Unlike reversed phase HPLC, the identification of the correct column chemistry is critical for the successful application of achiral pcSFC. Very different selectivity can be achieved depending on the column chemistry. Basic, neutral, and acidic compounds are well eluted on most columns that indicates the suitability of pcSFC for a broad range of chemical functionalities. The number of "SFC" columns for achiral purifications has also grown rapidly in the past three years. Activity in (i) agricultural and clinical research, (ii) environmental remediation, (iii) food and polymer science, (iv) petrochemicals, and (v) biological chemistry immediately come to mind. Additional Chapters 9–12 have been introduced into the book since writing began that reflect numerous additional applications of pcSFC such as pharmaceuticals, petroleum, food, personal care products, and cannabis. Additional advantages of SFC are listed in Table 0.2.

0.2 Background for the Book

While there have been numerous books published concerning SFC as both monographs and edited volumes, there appear to be only two texts that have had teaching as a major emphasis. One, published in 1990, was edited by Milton L. Lee (Brigham Young University) and Karin E. Markides (Uppsala University, Sweden) and written by a committee of peers is entitled "Analytical Supercritical Fluid Chromatography and Extraction" [4]. For chromatographic discussion, this book focused almost entirely on wall coated open tubular capillary column SFC (otSFC), which is not widely performed today having been replaced almost 100% by packed column SFC (pcSFC).

In the early days, otSFC and pcSFC coevolved and vigorously competed with each other as described in Chapter 1. otSFC lost ground and eventually faded

Table 0.2 Additional advantages using pcSFC.

- No pre-derivatization to achieve solubility and/or volatility
- Shorter cycle time with gradual gradient elution
- Faster separation facilitated by higher fluid diffusivity
- Reduced column diameter/particle size via lower fluid viscosity
- Less extreme chromatography conditions
- Routine normal phase chromatography

away, mainly as a result of poor chromatographic reproducibility issues in terms of flow rate, gradient delivery, pressure programming, and sample injection. The early systems were costly and not user friendly, which resulted in the technique being marginalized as too expensive and inefficient. While otSFC was capable of outstanding feats such as the separation of nonvolatile polymeric mixtures and isomeric polyaromatic hydrocarbons, most workers in the field would agree nowadays that the approaches used in otSFC are among the worst parameters to test with pcSFC.

Another book entitled "Packed Column SFC," published by the Royal Society of Chemistry and authored by Terry A. Berger [5] was published in 1995. Given that over 20 years have elapsed since the publication of Berger's book, the book presented here today provides ample references that reflect the current state-of-the-art as understood today. We have written our book that incorporates a more pedagogical style with the explicit intention of providing a sound education in pcSFC. Relatively new users of SFC in the early days were largely forced to rely on concepts developed for either HPLC (in the case of packed columns) or GC (in the case of open tubular columns), which were often inappropriate or misleading when applied to both otSFC and pcSFC. Our book addresses these deficiencies.

In this regard, a detailed discussion of current SFC instrumentation as it relates to greater robustness, better reproducibility, and enhanced analytical sensitivity is a focus of the book (Chapter 3). Originally, SFC was thought to be solely for low molecular weight, nonpolar compounds. Today, we know that SFC spans a much larger polarity and molecular mass range. Even though modern pcSFC books may be more adequately described as either "Carbon Dioxide-Based HPLC" (as Terry Burger once suggested) or "Separations Facilitated by Carbon Dioxide" (as suggested by Fiona Geiser) than "Packed Column Supercritical Fluid Chromatography," a change in nomenclature this drastic was not encouraged by attendees at several recent pcSFC conferences in both Europe and the United States. Suffice it to say, a change in nomenclature at this time is not suggested here. Nevertheless, this drastic shift in mindset and practice as suggested by Berger and Geiser during the last decade concerning both stationary phase and mobile phase has been a large reason for the current resurgence of pcSFC technology for problem solving at the industrial and academic levels worldwide. As proof, analytical scale achiral SFC is discussed in Chapter 6 along with ion pair SFC, reversed phase SFC, and HILIC-SFC.

While SFC has experienced much painful growth and disappointment during its evolution over 50 plus years, the "flame" has never been extinguished in the minds of a core group of separation scientists. A major reason for this mindset has been the near-annual, well-attended scientific meetings that have taken place in Europe and the United States over the past 25 years. Initially, the meetings were known as "the International Symposium on Supercritical Fluid

Chromatography and Extraction" wherein the focus was almost exclusively on capillary column SFC. Milton Lee at BYU and Karen Markides from University of Uppsala, Sweden served as hosts for the first meeting (1988) in Park City, UT. Subsequent meetings and approximate dates that have mostly been within the United States are listed in Table 0.3. Not shown in the table, but the youngest of us (DP) presented a poster at probably the earliest conference in this series called "SFC-87, Pittsburgh." Attendance was approximately 150.

These meetings were terminated soon after 2004 due to a lack of vendor commitment and support and user interest. In 2007, a series of new conferences with a different name ("International Conference on Packed Column Supercritical Fluid Chromatography") that gave attention to exclusively packed column Supercritical Fluid Chromatography was initiated first by Suprex Corporation, Pittsburgh, PA, then Berger SFC, and later by both Waters Corp. and Agilent. These meetings which now attract primarily industrial scientists, engineers, and academic colleagues from Europe and the United States are currently sponsored by the Green Chemistry Group. During the past 10 years the meetings have occurred annually and have alternated mostly between Europe and the United States (Table 0.4). To gain a greater world-wide audience the Green Chemistry Group has sponsored pcSFC meetings in China and Japan (i.e. 2016–2017, respectively). Additional meetings are scheduled in 2019 for both China and Japan.

pcSFC during the past 10 years has become a viable chiral chromatographic technique in the areas of pharmaceutical drug discovery and drug development. Chiral separations using carbon dioxide which incorporate a host of normal phase, silica-based stationary phases with principally ultraviolet and mass spectrometric online detection are now common. Nearly every pharmaceutical company in the United States, Asia, and Europe has multiple pcSFC instruments operating in a variety of laboratories. Interest in India, China, Korea, and the Pacific Rim, for example, is growing.

Table 0.3 Open tubular column SFC meetings.

SFC-1(1988) – Park City, UT
SFC-2 (1989) – Snowbird, UT
SFC-3 (1991) – Park City, UT
SFC-4 (1992) – Cincinnati, OH
SFC-5 (1994) – Baltimore, MD
SFC-6 (1995) – Uppsala, Sweden
SFC-7 (1996) – Indianapolis, IN
SFC-8 (1998) – St. Louis, MO
SFC-9 (1999) – Munich, Germany
SFC-10 (2001) – Myrtle Beach, SC
SFC-11 (2004) – Pittsburgh, PA

Table 0.4 Packed column SFC meetings.

pcSFC 2007 – Pittsburgh, PA, USA
pcSFC 2008 – Zurich, Switzerland
pcSFC 2009 – Philadelphia, PA, USA
pcSFC 2010 – Stockholm, Sweden
pcSFC 2011 – New York City, USA
pcSFC 2012 – Brussels, Belgium
pcSFC 2013 – Boston, MA, USA
pcSFC 2014 – Basel, Switzerland
pcSFC 2015 – Philadelphia, PA, USA
pcSFC 2016 – Vienna, Austria
pcSFC 2017 – Rockville, MD, USA
pcSFC 2018 – Strasbourg, France

Currently activity centers around (i) development and application of mass-directed pcSFC, (ii) enhancement of robustness and sensitivity to meet various regulatory requirements, (iii) production of new polar stationary phases for separation of metabolomics and related biochemicals, and (iv) theoretical modeling of column physical properties dictated by employment of compressible polar modified mobile phase and stationary phase – bonded sub-2-μm particles.

There is rapidly growing interest in achiral pcSFC where the separation of highly polar compounds has been demonstrated. Applications to polymeric materials, natural products, water soluble analytes, surfactants, organic salts, fatty acids, lipids, organometallics, etc. are experiencing great success. Depending upon the nature of the stationary and mobile phases employed, a variety of separation mechanisms can be expected such as reversed phase pcSFC, ion pairing pcSFC, and aqueous promoted HILIC-pcSFC. Each mode of chromatography can be expected to augment the more popular normal phase pcSFC that has been used for decades and employs nonpolar mobile phases.

0.3 Audience for the Book

This book will be of interest to industrial, government, and academic users of pcSFC and is expected to be useful as a chemistry textbook in graduate-level separations courses. Laboratories looking to adopt SFC as part of their regular analytical tools will find this book useful as they learn fundamental principles behind technology and how pcSFC complements both HPLC and GC.

One's view of SFC today is entirely different from that of 25–30 years ago wherein (i) flow rates and gradient delivery were not reproducible, (ii) analytical UV sensitivity was not acceptable, and (iii) stationary phases were designed

for reversed phase chromatography as opposed to normal phase chromatography. Today, SFC is considered to be primarily normal phase chromatography (i.e. a separation technique similar to HPLC) 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 with gradient elution where the composition of the mobile phase becomes more polar with time. Polar stationary phases such as bare silica, cyanopropyl-silica, 3-aminopropylsilica, and 2-ethylpyridylsilica are routinely employed. pcSFC has numerous practical advantages relative to reversed phase HPLC such as higher speed, greater throughput, more rapid equilibration, and shorter cycle times. SFC yields lower operating cost and lower column pressure drop, and is orthogonal to reversed phase HPLC. Finally, compounds of interest can be isolated with a relatively small amount of solvent because CO_2 vaporizes away. This feature has become particularly important for preparative applications in which elution volumes can be large.

During this time period, a SFC system was introduced by Waters Corp. (Milford, MA, USA). The system featured the efficient cooling of the CO_2 pump heads by Peltier and the design of a dual stage back pressure regulator that was heated to avoid frost formation. In this case, separations with the Waters instrument were mostly identified as ultrahigh performance supercritical fluid chromatography (UHPSFC). A similar system like Waters was introduced in 2012 by Agilent which was a hybrid that allowed both UHPLC and SFC separations. Shimadzu has more recently introduced hardware that performs similar operations. This combined vendor news reenergized many workers in the SFC community and caused potential users of the technology to re-investigate the research potential of pcSFC. The instrumentation from these three vendors nowadays appears to represent the current methodology to perform analytical pcSFC which should enhance its acceptability by the separation scientists into the immediate future. UHPSFC via either vendor affords a high throughput approach for profiling analytes such as free fatty acids, acylglycerols, biodiesel, peptides, basic drugs, etc. via light scattering, UV, and Q-TOF-MS detection without the waste and uncertainty of sample preparation procedures. This more modern terminology is prevalent throughout this book. The older pcSFC instruments, while still useable in numerous laboratories are no longer being manufactured.

0.4 SFC Today

Being green is a good thing, but most people nowadays seemingly go for pcSFC because of its speed and fast method development rather than its environmental advantages. Experts in the field now readily agree that ultrahigh performance supercritical fluid chromatography (UHPSFC) has established itself as

the preferred way of doing chiral and achiral analysis on both analytical and preparative scales. They also say that SFC will become the norm for small-scale purifications. Increased interest in (i) petrochemical and food industries, (ii) environmental air quality, (iii) biodiesel quality control, and (iv) protein separations can be expected in the not too distant future [6].

Much of the increased experimental capability alluded to above has been made possible by the introduction of pumping systems that deliver enhanced reproducible and accurate flow of CO_2 and modifier. In this case, separations are generally identified as UHPSFC.

Anyone with an interest in analytical and/or preparative scale pcSFC coupled to both spectroscopic and flame-based detectors will find this book beneficial. Subcritical fluid chromatography and enhanced fluidity chromatography as developed by Susan Olesik at the Ohio State University are also applicable here. Bonafide experience of the separation scientist in analytical or preparative scale SFC is not necessary for reading this book. Some knowledge of chromatographic principles is, however, desirable. With the introduction of more reliable instrumentation and eye-catching applications, a new generation of separation scientists and engineers are beginning to express much interest in the technology. Because the book is written with teaching in mind, the text could very well be the reference document on the desk of each person who is applying pcSFC.

Enjoy reading!
Larry M. Miller, J. David Pinkston, Larry T. Taylor
February, 2019

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1

Historical Development of SFC

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
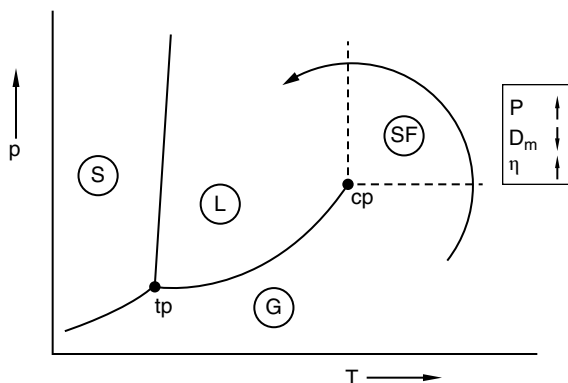
Figure 1.1 Phase diagram for a single pure component, illustrating areas in which solid (S), liquid (L), gaseous (G), and supercritical (SF) conditions occur. tp is the triple point and cp is the critical point. A gas can be transferred into a liquid by following the arrow. In doing so, the density, 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.
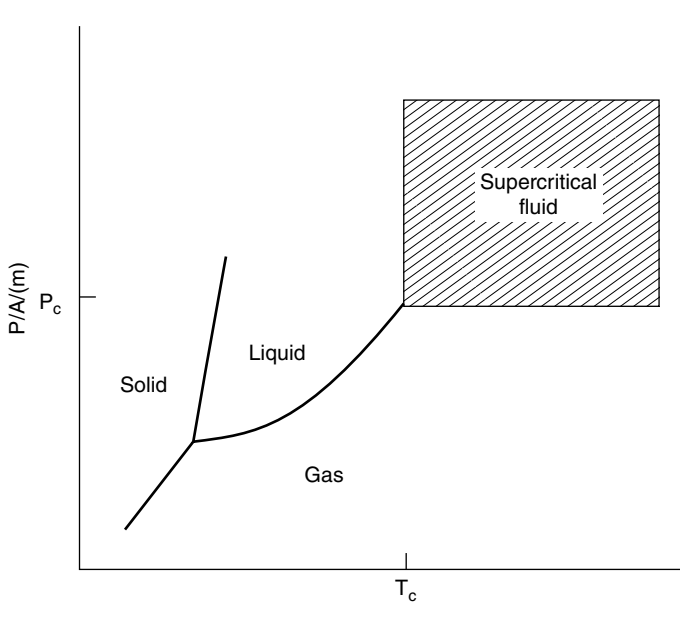
Figure 1.2 Definition of supercritical fluid by American Society for Testing and Materials (ASTM) and International Union of Pure and Applied Chemistry (IUPAC). *Source:* Smith [2]; ASTM [3]; Chester [4, vol. 2, p. 11, figure 2].
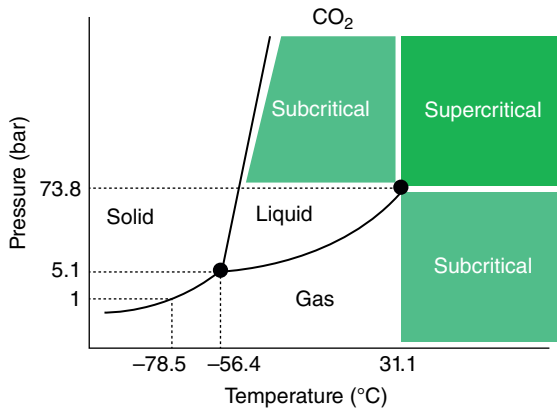
Figure 1.3 Misleading phase diagram for a single component supercritical fluid. *Source:* Laboureur et al. [5]. https://www.mdpi.com/1422-0067/16/6/13868. Licenced under CC BY 4.0.

   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
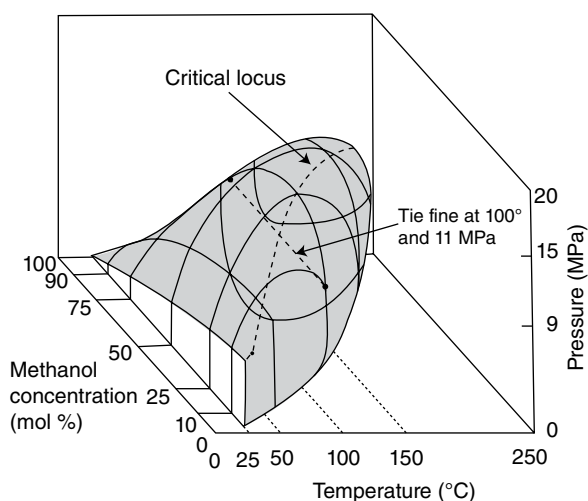
Figure 1.4 Two-phase *l-v* region of a binary mixture is a volume in a three-dimensional phase diagram. The Type I mixture CO_2-methanol is illustrated here. The two-phase region is the shaded interior of the figure. It has been cut off at 25 °C to show the isotherm, but actually extends to lower temperatures. *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)
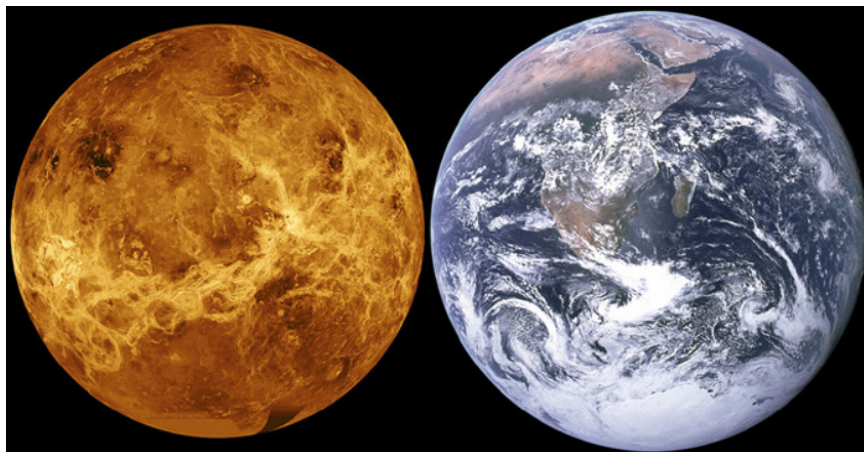- Zero surface tension
- Tunable solvent strength
- Nontoxic if CO_2

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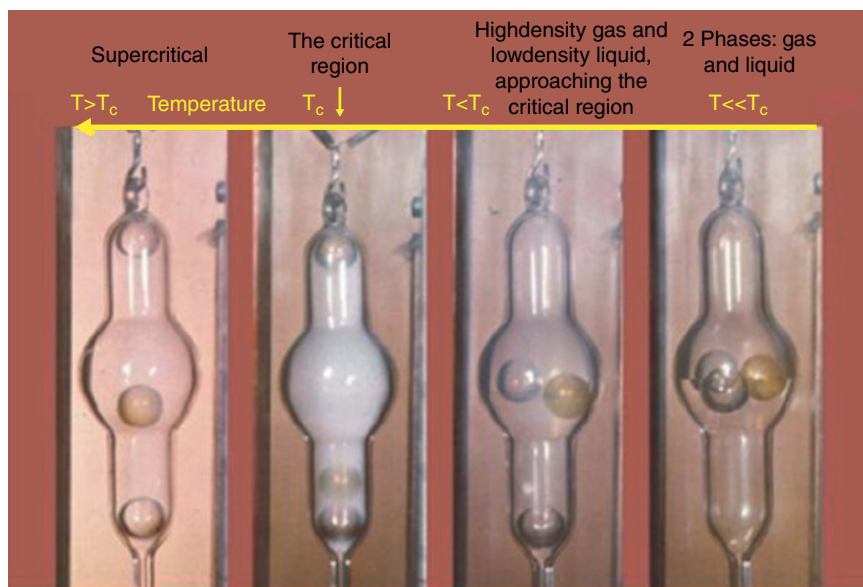
Figure 1.6 Behavior of four spheres of different densities in CO_2: supercritical, critical, subcritical, and liquid (0.92 g/mL). *Source:* Chemical & Engineering News, June 10, 1968, p. 105, Photo by Ray Rakow.

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